IMPACT OF UV RADIATION ON PLANT GROWTH AND DEVELOPMENT IN DIFFERENT CLIMATIC REGIMES

Effekter av UV-stråling på vekst og utvikling hos planter dyrket i ulike klimaregimer

Philosophiae Doctor (PhD) Thesis

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List of Papers

Paper I: UV-B–inhibition of stem elongation and leaf expansion in pea is associated with altered GA_1 metabolism in apical stem tissue and altered GA and IAA metabolism in young leaves.

Paper II: UV-B signaling in pea involves LONG1 and LIP1, homologs of *Arabidopsis thaliana* HY5 and COP1.

Paper III: The impact of UV radiation at high altitudes close to the equator on morphology and productivity of pea (*Pisum sativum* cv. Cascadia) in different seasons.

Paper IV: Effect of UV radiation on the growth and postharvest characteristics of three pot-rose cultivars grown at different altitudes.

Paper V: Growth and morphology of pea (*Pisum sativum* cv. Oregon sugar pod II) grown under different shading screens in Ethiopian climate condition.

Abbreviations

ABA	Abscisic acid
COP1	CONSTITUTIVE PHOTOMORPHOGENIC 1
CPD	Cyclobutane pyrimidine dimer
DNA	Deoxyribonucleic acid
GAs	Gibberellic acids
HY5	ELONGATED HYPOCOTYL5
HPLC	High-pressure liquid chromatography
IAA	Indole-3-acetic acid
LED	Light emitting diode
PAR	Photosynthetically active radiation
PSII	Photosystem II
qPCR	Quantitative real time Polymerase Chain Reaction
RH	Relative humidity
ROS	Reactive oxygen species
SLA	Specific leaf area
UV	Ultraviolet
UVR8	Ultraviolet resistance locus 8
VPD	Vapor pressure deficit

Abstract

Ultraviolet (UV) radiation has various effects on the growth, morphology, and biochemical composition of plants. Currently, there is an increasing interest in the manipulation of UV radiation in glasshouses, polythene tunnels, and other protected horticultural environments. In this study, UV radiation was manipulated by the use of UV lamps and filters with different UV-transmittance. The main objective was to study the effects of UV radiation under different climatic regimes in the regulation of plant growth and development, biomass production and yield, postharvest life, and the content of secondary metabolites and plant hormones.

In controlled growth-chambers, the effect of 6 h daily UV-B radiation provided by UV-B lamps and two temperature regimes (constant, 20℃ and temperature drop, 21 to 13℃) on the morphology, DNA damage, hormone physiology, and content of phenolic compounds were investigated in pea (*Pisum sativum* cv. Torsdag). UV-B exposure at 0.45 W m^{-2} for 10 days reduced shoot extension growth and leaf area by 9% and 30% respectively, when provided under a constant temperature (20℃). Under a daily temperature-drop treatment (21℃ to 13℃), the UV exposure reduced the shoot elongation and leaf area by 30% and 18%, respectively, as compared to a temperature drop only. Although the UV levels were not identical under the two temperature regimes because of reduced efficiency of the UV lamps under the temperature-drop treatment, shoot elongation was apparently more strongly affected by UV-B under the temperature drop than when provided under constant temperature. These morphological changes were associated with the reduced levels of the bioactive gibberellin GA_1 (54–69%) in apical stem tissue and young leaves. Also, reduction of the content of IAA in the young leaves (27–35%) was observed.

In *Arabidopsis thaliana,* COP1 and HY5 are central players in UV-B signaling resulting in formation of UV-B-protecting compounds and altered morphogenesis. In pea, we investigated the roles of the HY5 and COP1 homologs LONG1 and LIP1 in protection towards UV-B-related damage and altered morphogenesis. By the use of high performance liquid chromatpgraphy (HPLC), eighteen different chromatographic peaks of phenolic compounds were detected in pea leaves. However, the focus in this thesis is on the glycosides of the major flavonols, quercetin, kaempferol, and myricetin as well as two major flavones, luteolin and apigenin. Consistent with LONG1 and LIP1 as UV-B signaling compounds in pea, the *long1* and *lip1* mutants exhibited hypersensitivity and higher resistance to UV-B compared to the

wild type (WT), respectively, probably due to their lower and higher levels of specific flavonoid glycosides. Also, *long1* showed significantly higher levels of UV-related DNA-damage products (cyclobutane pyrimidine dimers (CPDs)) compared to WT plants. On the contrary, plants mutated in the *LIP1* gene, showed less DNA-damage and higher levels of individual phenolic compounds than the WT plants. The dwarfed *le* GA biosynthesis mutant and the elongated *la cry-s* GA signaling mutant, which behaves like being GA saturated, were both more resistant to UV-B-related damage than the WT, probably due to higher levels of specific flavonoid glycosides, as shown in *le*. GA_3 application did not affect the sensitivity to UV-B-related damage. These studies demonstrate that LONG1 and LIP are essential UV-B signaling components in pea, and that GA content and degree of extension growth do not affect susceptibility to UV-B-related damage.

By using UV-transmitting and UV-blocking films, the effect of natural levels of UV radiation on growth, morphology, and days to flowering of pea and pot-rose cultivars were evaluated at a higher (2800 masl) and lower altitude (1700 masl) in Ethiopia. At both altitudes, the pea and rose cultivars grown under the UV-transmitting film had shorter shoots and delayed flowering as compared to plants grown under the UV-blocking films. However, the UV-transmitting and UV-blocking films did not show differences in terms of the shelf life of pot-roses or pod production in pea. Regardless of UV-radiation, rose cultivars grown at the higher altitude had higher stomata conductance than the plants grown at the lower altitude. However, in pea the stomata conductance increased under UV-transmitting film at the higher altitude, showing that the stomatal response to UV is different depending on the background climate and plant species. In conclusion, UV radiation mainly affects plant morphology and flowering time, but climatic factors such as irradiance, temperature, and vapor pressure deficit (VPD) seem to have a stronger effect on the stomata conductance, postharvest water usage, and pea productivity.

Furthermore, different types of screens (Svensson reflective screen with strip ventilation and white plastic as well as one locally produced screen yellow plastic) were used as greenhouse covers to study their effects on the performance and productivity of pea (cv. Origan pod III) during the dry season in Ethiopia. The enhanced shoot elongation under the Svensson reflective screen as compared to the plastic films was mainly because of the reduced transmittance of photosynthetic active radiation (PAR) of the Svensson screen as compared to the plastic screens (white and yellow). However, the screening material did not have a significant effect on the pod production, which confirms that pea is robust to changes in light

quality. Reduced transpiration and lower water usage per pod were found under the locally produced yellow plastic film as compared to the imported screens.

Keywords: Auxin, flavonoid, gibberellin (GA_1), morphology, plant hormones, ultraviolet radiation (UV), screen

Sammendrag

Ultrafiolett (UV) stråling har effekt på planters vekst, morfologi og kjemisk innhold. Det er stor interesse for å manipulere UV stråling i kommersiell produksjon av planter i veksthus, plasttunneler og i andre typer dyrkingssystemer med kontrollert klima for å påvirke viktige planteprosesser. I dette arbeidet ble UV stråling manipulert ved filtrering av naturlig sollys gjennom ulike filter og ved bruk av UV lamper. Hovedformålet var å studere effekter av UV på plantevekst og utvikling, biomasseproduksjon og avling, holdbarhet, samt innholdet av sekundære-metabolitter og plantehormoner.

I kontrollerte vekstkammere ble effekten av 6 timer daglig UV-B eksponering studert under to ulike temperaturregimer; konstant temperatur (20℃) og temperatursenkning (fra 21 til 13℃) på morfologi, DNA skade, hormoninnhold, og innholdet fenoler hos ert (*Pisum sativum*) cv Torsdag. Kombinasjonen av konstant temperatur og UV-B eksponering (0.45 W m^{-2}) i 10 dager reduserte strekningsveksten og bladarealet med henholdsvis 9% og 30%. UV-B eksponering samtidig med temperatursenkning reduserte strekningsveksten og bladarealet med henholdsvis 30% og 18%. Selv om UV nivået ikke var likt i de to temperatur-regimene, fordi effektiviteten til UV lampene reduseres ved lavere temperatur, vister resultatet at UV-B virker svært hemmende på strekningsveksten om det gis samtidig med en temperatursenkning. De observerte morfologiske endringene viste en klar sammenheng med endringer i innholdet av plantehormoner som påvirker strekningsvekst. Innholdet av aktivt gibberellin (GA_1) var 54 -69% lavere i unge skudd og unge blad i planter eksponert for UV-B og temperatursenkning sammenlignet med de andre behandlingene. I tillegg ble det målt 27-35% lavere innhold av auxin i unge blad.

Hos *Arabidopsis thaliana* er COP1 og HY5 kjent som viktige komponenter i UV-B-signaleringen. I ert ble HY5 og COP1-homologene LONG1 og LIP1 studert for undersøke deres rolle i signalering knyttet til beskyttelse mot UV-B-skader og endringer i morfologi. Ved hjelp av væskekromatografi ble det identifisert 18 ulike fenolforbindelser i erteblad med antatt beskyttende funksjon. De dominerende fenolforbindelsene var glykosider av flavonolene quercetin, kaempferol og myricetin, og flavonene luteolin og apigenin. Mutantene *long1* and *lip1* viste henholdsvis hypersensitivitet og høyere motstandsdyktighet mot UV-B stråling

sammenlignet med villtypen (VT), sannsynligvis på grunn av lavere og høyere innhold av spesifikke flavonoider. Planter mutert i LONG1 viste også signifikant høyere nivå av UV-relatert DNA skade (cyclobutan pyrimidin dimere) mens planter mutert i *LIP1* genet viste mindre DNA skade sammenlignet med VT.

En dvergtype og av ert med lavt innhold av GA (*le*) og en GA signaleringsmutant (*la cry-s*) med sterk strekningsvekst på grunn av tilsynelatende mettet-GA respons, viste begge motstandsdyktighet mot UV-B sannsynligvis på grunn av et høyt innhold flavonoider. GA_3-applisering endret ikke erteplatenes følsomhet for UV-B-relater skade. Videre, viste ikke *long1*, *cry-s* og *le* mutanten redusert strekningsvekst ved UV-B-eksponering slik som VT. Arbeidet viser at LONG1 og LIP er sentrale komponenter i UV-B signaleringen hos ert, men at GA og strekningsvekstresponser antagelig ikke påvirker plantenes følsomhet for UV-B-relatert skade. Resultatene kan imidlertid tyde på at plantenes må ha evne til å kontrollere nivået eller responsen på GA for å kunne respondere på UV-B som en regulator av strekningsvekst.

Ved å benytte UV transmitterende og UV blokkerende film i ulike høyder over havet (2800-1700 moh) i Etiopia ble effekten av UV stråling undersøkt på vekst og avling hos ert og vekst og holdbarhet hos potteroser. I dette området nær ekvator finnes verdens høyeste nivåer av UV- stråling. Både ert og roser viste endringer i morfologi og blomstringstid og planter eksponert for UV (+UV) var kortere og viste forsinket blomstring sammenlignet med –UV uansett høyde over havet. Det var små forskjeller i avling hos ert og holdbarhet hos roser under de ulike behandlingene og tyder på at det er andre klimafaktorer enn UV som har størst effekt på disse egenskapene. Effekten av UV på konduktans varierte med planteslag og bakgrunnsklima. Det var ingen signifikant effekt av UV på vannforbruk under eller etter produksjon hos roser. Hos ert hadde UV ingen effekt på konduktans ved 1800 moh men ved 2800 moh førte UV-stråling til økt konduktans. Hovedkonklusjonen fra dette arbeidet er at UV har effekt på morfologi og blomstringstid men at andre klimafaktorer (temperatur, luftfuktighet og lysmengde) i større grad påvirker avling hos ert (antall erteskolmer) og holdbarhet hos roser.

Effekten av ulike typer skyggemateriale ble undersøkt i et forsøk med ert (cv. Origan pod III) i Etiopia. Vekst og avling ble sammenlignet under tre ulike typer skyggemateriale: rimelig lokalprodusert plastfilm, kostbar importert plastfilm og en svært kostbar ventilerende skyggeduk med reflekterende aluminium (Svensson). Plantene under Svensson viste økt strekningsvekst på grunn av lavere mengde fotosyntetisk aktivt lys. Det var imidlertid ingen forskjell i avling (biomasseproduksjon eller antall erteskolmer) mellom de ulike skyggematerialene og viser at ert er svært robust for endringer i lyskvalitet. Redusert

transpirasjon ble målt under den lokal-produserte plasten og viser at den kan være en aktuell skyggeduk å benytte i erteproduksjon også med tanke på effektivt vannforbruket i produksjonen.

1 Introduction

1.1 Solar UV radiation

Ultraviolet (UV) radiation is an integral part of the sunlight that reaches the surface of the Earth. The UV region of the spectrum is divided into three parts: UV-A (315–400 nm), UV-B (280–315 nm), and UV-C (less than 280 nm) (Li *et al.*, 2013; MORALES *et al.*, 2014). The energy of a photon is inversely related to its wavelength; therefore, UV-C radiation is the most energetic of the three wavebands (Paul & Gwynn-Jones, 2003). UV-C is strongly absorbed by the ozone in the atmosphere and does not penetrate through the atmosphere. However, most of the UV-A and part of the UV-B reach the earth's surface. UV-B accounts for less than 0.5% of the total light energy reaching the earth's surface, but it has the highest energy of the daylight spectrum (Jenkins, 2009; Heijde & Ulm, 2012).

The intensity of solar UV radiation reaching to the earth's surface varies based on different environmental factors such as the ozone layer, solar elevation, atmospheric composition, clearness of the sky, time of the day, and altitude (Madronich *et al.*, 1998). There is a strong effect because of changes in latitude, altitude, season, and time of the day, being highest in the tropics, especially at high altitudes in the summer at noon (Blumthaler *et al.*, 1992). The UV irradiance increases with altitude because the amount of absorbers in the overlapping atmosphere decreases with increasing altitude. Various reports have indicated that UV radiation increases 6–8% per 1000 m increase in altitude (Vanicek *et al.*, 2000). On a global basis, the weighted daily UV-B irradiance received at low latitude, high elevation sites can be nearly six times greater than the maximum dose received at arctic latitudes (Caldwell *et al.*, 1980). Clouds influence the UV reaching the ground surfaces through reflection, absorption, and scattering in to the atmosphere. A complete light cloud cover prevents about 50% UV radiation energy from reaching the earth's surface (Diffey, 1991).

1.2 Sensing the light

The ability of plants to sense and respond to light depends on their photosynthetic pigments and photoreceptors that absorb different wavelengths of the light. Responses to light quality such as far-red (FR) (700–800 nm) and red (R) light (600–700 nm) depend on the light absorbing pigment phytochrome, which senses the relative amount of R and FR light in the environment (Smith, 2000). Blue (B) light (400–500 nm) is absorbed not only by phytochrome but also by the B/UV-A absorbing pigment cryptochrome and phototropin (Lin, 2000). In *Arabidopsis thaliana*, UV RESISTANCE LOCUS 8 (UVR8) has been recently identified as a photoreceptor that detects UV-B radiation (Rizzini *et al.,* 2011; Christie *et al.,* 2012). UV-B perception by UVR8 is mediated by tryptophan-285 (Trp-285) and tryptophan-233 (Trp-233), which directly absorb and are excited by UV-B (Tilbrook *et al.*, 2013). UVR8 is built up as a seven–bladed *β*-propeller protein which is present both in the cytoplasm and the nucleus (Brown *et al.,* 2005) and with nuclear enrichment under UV-B exposure (Kaiserli & Jenkins, 2007; Jenkins, 2009). Plant perception of UV-B radiation as an environmental stimulus is known to affect growth and development (Jenkins, 2009). However, UVR8 must be associated with a molecular signaling pathway for UV-B perception to be translated into plant responses. Different reports have indicated that UVR8 interacts with the transcription factors ELONGATED HYPOCOTYL 5 (HY5) and CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1) in the nucleus in the presence of UV-B and visible light (Osterlund *et al.*, 2000; Yi & Deng, 2005; Oravecz *et al.,* 2006). There is evidence that COP1 and HY5 both play major roles in promoting UV-B–induced photomorphogenesis (Fig. 1) (Heijde & Ulm, 2012).

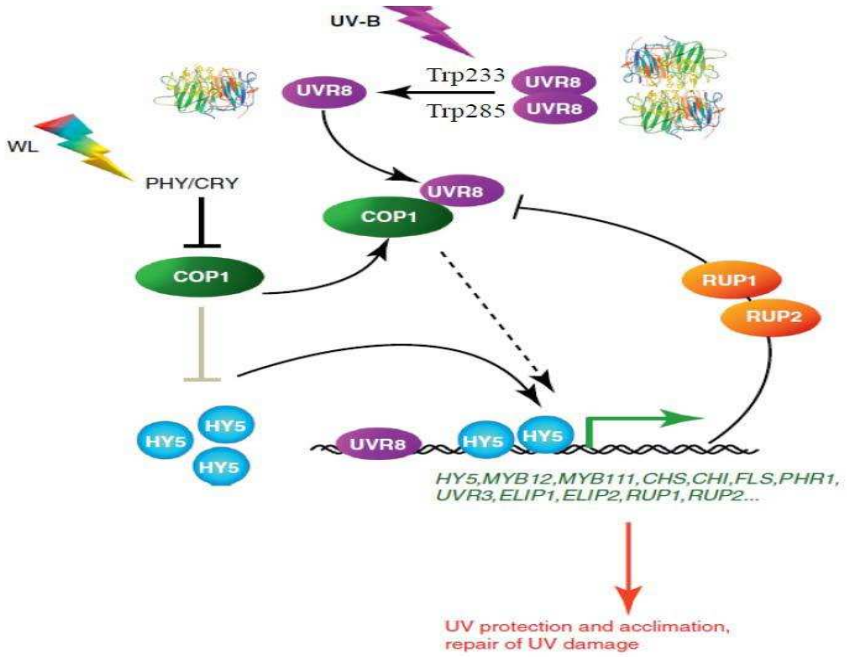

Fig. 1. Model of UVR8-mediated signaling. Under light (white light; WL) conditions devoid of UV-B, UVR8 is present mainly as a homodimer. COP1 represses photomorphogenesis by promoting degradation of HY5 (and other promotive transcription factors), but is under the negative control of light-activated phytochromes and cryptochromes. In the presence of UV-B radiation, UVR8 monomerizes and interacts with COP1. The bZIP transcription factor HY5 is stabilized and UV-B–responsive genes are activated. These include genes encoding proteins of importance for UV protection (e.g. phenylpropanoid biosynthesis pathway, including CHS and FLS) and DNA damage repair (e.g. photolyases PHR1 and UVR3), but also the RUP1 and RUP2 proteins, which constitute negative feedback on UVR8 activity involving direct protein–protein interaction (Heijde & Ulm, 2012; Tilbrook *et al.*, 2013).

Molecular analysis has shown that COP1 and HY5 are the major downstream effectors in UV-B responses as well as in visible light signaling, indicating high potential for cross-talk between UV-B and visible light responses (Heijde & Ulm, 2012). COP1 acts as a positive regulator of photomorphogenic UV-B response in *Arabidopsis thaliana*, whereas it function as a repressor in visible light-induced photomorphogenesis (Oravecz *et al.*, 2006; Heijde & Ulm, 2012). Moreover, it has been shown that in light-conditions lacking UV-B, the UVR8 photoreceptor exists as a homodimer (inactive dimer), which undergoes instant

monomerization (active monomer) following UV-B exposure and the process is meditated by the Trp-285 or Trp-233 amino acids (Christie *et al.*, 2012; Wu *et al.*, 2012; Tilbrook *et al.*, 2013) (Fig. 1). The active UVR8 monomers interact with the E3 ubiquitin ligase COP1 (Tilbrook *et al.*, 2013). Visible light activation of photoreceptors leads to the inactivation and nuclear exclusion of COP1, allowing HY5 stabilization and activation of light responsive genes (Osterlund *et al.*, 2000). In darkness, COP1 targets HY5 for ubiquitination and degradation, leading to suppression of photomorphogenesis (Saijo *et al.*, 2003). On the contrary, white light supplemented with UV-B radiation induces nuclear accumulation of both COP1 and HY5, and due to the inactivation of the COP1 ubiquitin ligase activity upon the COP1-UVR8 interaction, HY5 is not targeted for degradation of by COP1 (Oravecz *et al.*, 2006).

1.3 UV–B and plant responses

UV-B radiation is a key environmental signal that initiates diverse responses in plants, including metabolism, growth, and development. Exposure of plants to rather high levels of UV-B radiation might reduce photosynthesis (Dai *et al.*, 1992). At lower irradiance, UV-B radiation induces morphological changes such as reduction in shoot elongation and leaf area, changes in plant architecture, and accumulation of UV-B–absorbing compounds (Jenkins, 2009; Torre *et al.*, 2012). Although the level of UV-B radiation and plant adaptation influence the sensitivity of plants to UV-B radiation, it is well known that the sensitivity to UV-B radiation is dependent on different environmental factors such as drought, photosynthetic photon flux density (PPFD), and temperature (Mirecki & Teramura, 1984; Murali & Teramura, 1986; Sullivan & Teramura, 1990; Mark & Tevini, 1996).

Plants distributed along high elevations, where UV-B fluence is high, have a more pronounced adaptive mechanism than those at lower elevations (Sullivan *et al.*, 1992; Jansen *et al.*, 1998). Different genotypes within a species may also differ in their tolerance and response to UV-B. Increasing UV-B radiation can also stimulate the protective mechanism in plants, leading to modulation of the sensitivity of the photosynthetic apparatus to UV-B (Jansen *et al.*, 1998; Lavola, 1998). Based on the growth conditions and geographic location, there is a large variation among plant species when it comes to UV sensitivity. Commonly, UV-B tolerance in plants increases with increasing altitude (Ziska *et al.*, 1992).

Tolerance to UV-B is also a question of the balance between damage, repair, and acclimation (Frohnmeyer & Staiger, 2003). Plant species, which are faster in repairing the damaged DNA, are more tolerant to UV-B–induced stresses. Plants efficiently repair UV-B–induced DNA damage by a photoreactivation mechanism. This process is mediated by UV-A and blue light where the enzyme photolyase breaks the chemical bonds of cyclobutane rings and reverts the damage (Jansen *et al.*, 1998) (Fig. 2).

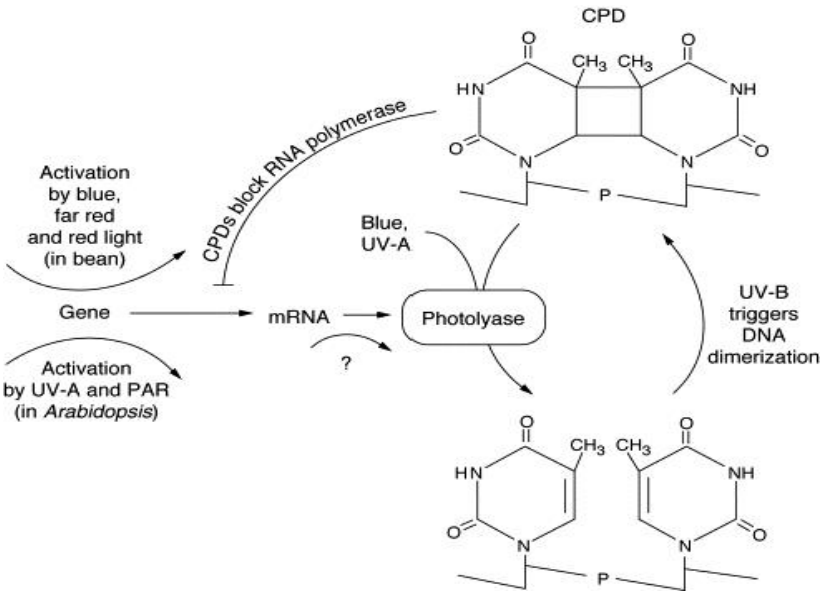

Fig. 2. Diagram showing the regulation of cyclobutane-pyrimidine dimer (CPD) photoreactivation. Transcription of genes encoding photolyases is minimal in the dark, but induced by blue, far-red, and red wavelengths, possibly involving phytochrome (Jansen *et al.*, 1998).

The production of reactive oxygen species (ROS) and the associated oxidative damage has also been observed in plants exposed to high UV-B doses. ROS are a by-product formed as a result of successive electron reduction of molecular oxygen (O_2), and they include the superoxide radical ($\cdot O_2^-$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($\cdot OH$) (Bolwell

& Wojtaszek, 1997). Hydroxyl radicals, singlet oxygen, superoxide radicals, and hydrogen peroxide are among the main ROS produced by UV-B (Soheila, 2000; Brosché & Strid, 2003). However, ROS are not only a source of cellular damage but also important signaling molecules that regulate the expression of several UV-B–responsive genes (Soheila, 2000; A-H-Mackerness *et al.*, 2001).

1.4 Induction of phytochemicals

Light is one of the best known environmental factors affecting the phytochemical composition of plants (Shohael *et al.*, 2006; Pérez-Balibrea *et al.*, 2008). High UV-B dosage and longer time of exposure enhance flavonoid biosynthesis and increase the concentration of phenolic compounds in plants (Treutter, 2005). The levels of UV-B–absorbing phytochemicals are well known to increase with increasing UV-B doses (Karousou *et al.*, 1998; Johnson *et al.*, 1999). Thus, UV-B exposure contributes to the accumulation of phenolic compounds in plant tissue as a protective response against UV radiation (Jansen *et al.*, 1998). However, different compounds show different efficiency in UV protection. A study on *A. thaliana* indicated that sinapate esters are more effective in preventing UV-B injury than the flavonoid derivatives (Landry *et al.*, 1995; Sheahan, 1996). This indicates that the protection against UV-B depends on the plant species and the types of phytochemicals produced.

Genotypes lacking the accumulation of secondary metabolites such as flavonoids are highly UV sensitive (Landry *et al.*, 1995). Leaf curling is a characteristic response to UV-B, and is considered a morphological adjustment to protect plants from the UV-B radiation through reduction in exposure area. Upward leaf curling in response to UV-B exposure suggests the UV-B–induced inhibition of cell division or more expansion on the upper side of the leaves compared to the lower side (Landry *et al.*, 1995; Greenberg *et al.*, 1997; Jansen *et al.*, 1998). Previous studies have indicated that the *uvr8* mutant is susceptible to UV-B–induced damage such as curled and chlorotic leaves, suggesting a lower content of UV-screening compounds in the mutant (Favory *et al.*, 2009). This confirmed the role of UVR8 in the regulation of genes responsible for biosynthesis of secondary metabolites (Morales *et al.*, 2013).

1.5 Regulation of plant hormone biosynthesis

Plant hormones are chemical messengers that coordinate the growth and development of plants. They are a collection of small molecules that at very low concentrations integrate environmental stimuli with plant cellular activity. Plant hormones regulate every aspect of plant growth and development from the cellular level to the stage of organogenesis (Stamm & Kumar, 2010). The major classes of plant hormones are gibberellins (GAs), auxins (IAA), abscisic acid (ABA), cytokinins (CK), ethylene, brassionosteriod, salicylic acid, jasmonate, and strigolactone.

1.5.1 Gibberellin biosynthesis and inactivation

Gibberellins (GAs) are a group of diterpenoid acids that function as growth regulators of plants influencing a range of developmental processes in higher plants including stem elongation, germination, dormancy, flowering, sex expression, enzyme induction, and leaf and fruit senescence.

GA biosynthesis is mainly affected by tissue type, developmental stage, light, temperature, and endogenous feed-back and feed-forward responses to GAs (Kamiya & García-Martínez, 1999; Hedden & Phillips, 2000). It has been reported that changes in stem elongation in pea (*Pisum sativum*) in response to alteration in day and night temperatures or exposure to a temperature drop during the day is related to the changes in endogenous level of GA_1 (Grindal *et al.*, 1998). GA_1 is the major active GA regulating stem length in pea (Ingram *et al.*, 1984). GA_1 is synthesized by the conversion of GA_{20} to GA_1 and catalyzed by GA3-oxidase (GA3ox) which is encoded by the *LE* gene (Fig. 3) (Campell & Bonner, 1986; Lester *et al.*, 1997; Weller *et al.*, 2009; Reinecke *et al.*, 2013).

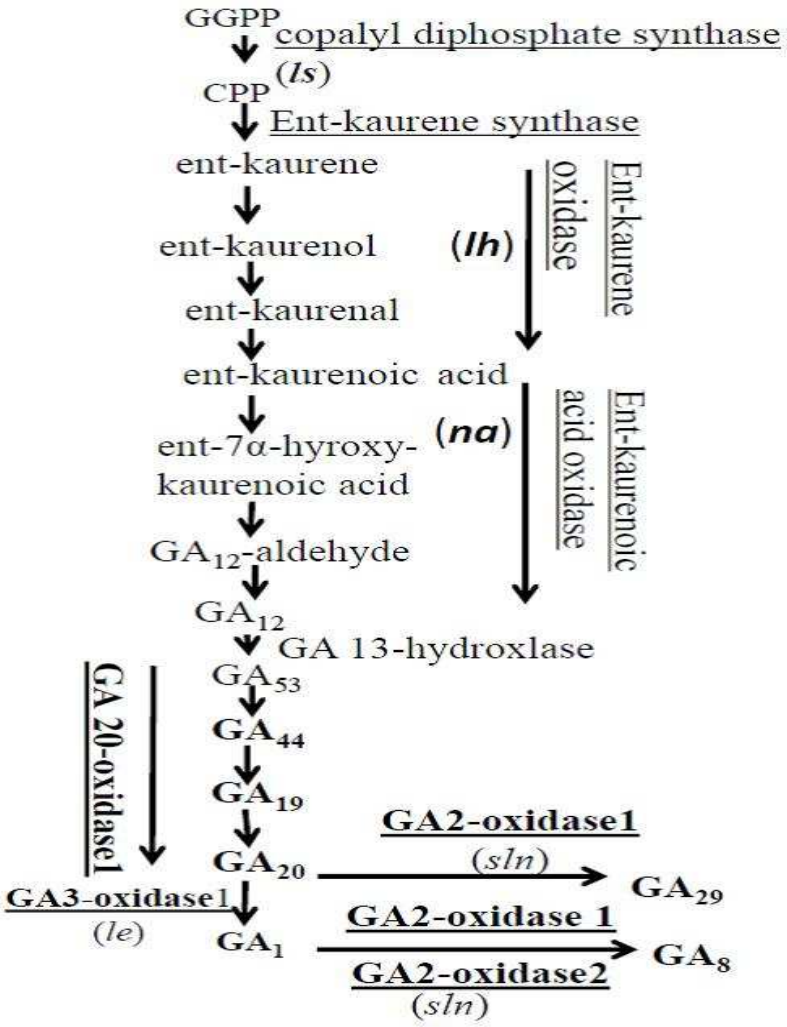

Fig. 3. Schematic representation of 13-hydroxylation pathways of gibberellin biosynthesis in vegetative tissue of pea. The enzymes cloned and characterized in pea are underlined. Corresponding mutants are given in parenthesis(Stavang *et al.*, 2005).

The different genes involved in the conversion process during the early and late stage of GA biosynthesis mainly affect the accumulation of bioactive GA_1 in pea shoots. These genes include *LS, LH and, NA* , *GA20ox1 and GA3ox1* (*LE*) and the two GA deactivation 2-oxidases (*SLN*, slender), *GA2ox1* and *GA2ox2* (Elliott *et al.*, 2001; Stavang *et al.*, 2005). The *GA2ox1* gene metabolizes the 2-oxidation of GA_{20} to GA_{29} and to GA_{29} catabolite, and the 2-oxidation of GA_1 to GA_8, while the *GA2ox2* gene product has a strong preference for GA_1 rather than GA_{20} as substrate (Reid *et al.*, 1992; Lester *et al.*, 1999). In *A. thaliana*, up-regulation of

GA2ox1 in response to UV-B was demonstrated recently, and a model was presented for the mechanism of UV-B action in this respect (Fig. 4) (Hayes *et al.*, 2014)

It has been reported that light and plant photoreceptors modulate the expression of genes responsible for hormone biosynthesis (Folta *et al.*, 2003; Weller *et al.*, 2009). In pea, light-induced interaction of *LIP1* and *LONG1*, the pea orthologs of *A. thaliana* COP1 and HY5, respectively, regulate the expression of the GA catabolism gene *GA2ox2* and thus the level of bioactive GA_1 (Weller *et al.*, 2009; Li & Huang, 2011). The *long1* mutant maintains high GA levels under light because of greatly reduced light-induced expression of *GA2ox2*. Thus, it appears that LONG1 is required to activate *GA2ox2* transcription and thus decrease the GA levels after transfer to light (Weller *et al.*, 2009; Lau & Deng, 2010). Light-induced regulation of GA biosynthesis in germinating *A. thaliana* seeds appears to be achieved through the degradation of the basic-helix-loop-helix (bHLH) transcription factor PHYTOCHROME INTERACTING FACTOR 1/PHYTOCHROME INTERACTING FACTOR-LIKE 5 (*PIF1/PIL5*), which also acts in the repression of GA biosynthesis genes (Oh *et al.*, 2006). As PIF proteins are bound to and targeted for degradation by activated phytochromes, light activates GA biosynthesis through repression of a repressor (Castillon *et al.*, 2007).

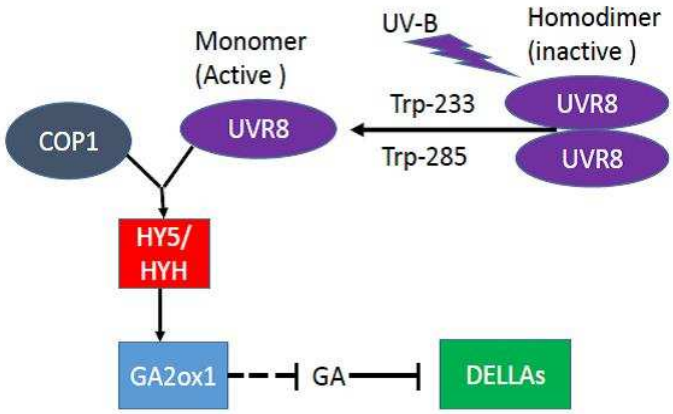

Fig. 4. Hypothesized role of UV-B in GA regulation in *Arabidopsis thaliana*. UVR8 photoreceptor exists as homodimer (inactive dimer) which undergoes instant monomerization (active monomer) following UV-B exposure and mediated by the Trp-285 and Trp-233 amino acids. UV-B perceived by the photoreceptor UVR8 interacts with *COP1* and up-regulates transcription of *HY5* and *HYH*. GA2ox1 levels increase, resulting in reduced GA levels, and increased *DELLA* stability (Heijde & Ulm, 2012; Hayes *et al.*, 2014a).

1.5.2 Auxin biosynthesis and inactivation

Indole-3-acetic acid (IAA) is one of the naturally occurring growth hormones, which enhance cell division, cell elongation, cell differentiation, tropism, and flower development. IAA biosynthesis in plants is very complex and not well understood because of the existence of multiple pathways, involvement of many genes, and the impact of environmental factors. Genetic and biochemical studies have indicated that tryptophan (Trp) is a main precursor for IAA in plants (Woodward & Bartel, 2005; Zhao, 2010). At least four different pathways for Trp-dependent biosynthesis of IAA in plants have been proposed: the *YUCCA* (YUC) pathway, the indole-3-pyruvic acid (IPA) pathway, the indole-3-acetic amide (IAM) pathway, and the indole-3-acetaldoxime (IAOx) pathway (Tivendale *et al.*, 2010; Mashiguchi *et al.*, 2011). Among the pathways, the *YUC* pathway has been reported as a common IAA biosynthesis pathway in various plants (Cheng *et al.*, 2006; Yamamoto *et al.*, 2007). In pea, two *YUC* genes, *YUC1* and *YUC2*, have been reported (Tivendale *et al.*, 2010; Li *et al.*, 2012).

High R:FR (ratio of red to far-red light) acts in the blocking of the transcription of IAA-biosynthesis genes through phosphorylating PIF transcription factors and the inactive form of PHYB (Pfr) in the nucleus, whereas low R:FR acts in an opposite manner (Li *et al.*, 2012). Moreover, high irradiance of white light photo-oxidizes IAA and inhibits cell growth in *in vitro* culture and seed germination in pea (Fukuyama & Moyed, 1964). It has also been shown that plants exposed to R light also have lower mobility of IAA in the cell because of the lower rate of biosynthesis and thus lower IAA levels (Iino, 1982). IAA is well known to regulate phototropism in the plant. However, exposure of plants to UV-B radiation partially results in loss of their responses to phototropism (Ros & Tevini, 1995). Such loss of phototropism response might be related to the UV-B–induced degradation of IAA. UV-induced IAA degradation might be related to the ability of IAA to absorb the UV wavelengths from 270 to 300 nm (Ros & Tevini, 1995; Krizek *et al.*, 1997). Moreover, UV-B radiation has been shown to lower the concentration of IAA in various plant species such as fronds (*Spirodela oligorbiza*), cucumber (*Cucumis sativus* L), rice (*Oryza sativa* L.) and *A. thaliana* (Witztum *et al.*, 1978; Huang *et al.*, 1997; Krizek *et al.*, 1997; Hectors *et al.*, 2012)

1.5.3 ABA and ABA metabolites

The plant hormone ABA serves as an endogenous messenger that plays a key role in the growth and development of plants in response to environmental stimuli (Raghavendra *et al.*, 2010). In addition to its growth regulatory role, ABA is considered as essential messenger involved in the adaptive responses of plants against abiotic and biotic stresses (Umezawa *et al.*, 2006; Danquah *et al.*, 2014). In green plants, ABA biosynthesis starts with isopentenyl diphosphate (IPP) and occurs via the carotenoid violaxanthin (Taiz & Zeiger, 2010). Environmental factors such as drought stress, flooding, UV radiation, and some other biotic factors all play a role in regulating the ABA content in the plant (Zabadal, 1974; Atkinson & Urwin, 2012). Studies of the effects of light quality indicate that grape skin treated with R light has a higher concentration of ABA in the skin than those treated with B light or the control grape fruit skin (Kondo *et al.*, 2014). Moreover, irradiation of maize (*Zea mays* L) leaves with 3.3 W m^{-2} UV-B radiation for 4 h increased the level of ABA by 50 ng g^{-1} fresh weight compared to control (Tossi *et al.*, 2009).

1.6 The role of UV in horticultural industry

Currently, there is an increasing interest in the manipulation of UV radiation in glasshouses, polythene tunnels, and other protected horticultural environments. Most horticultural glazing materials block UV-B and UV-A wavelengths shorter than 360 nm (Paul & Gwynn-Jones, 2003). The absence of such wavelengths might result in enhanced shoot elongation and reduced branching, which are undesirable commercially. Efforts have been made to regulate plant growth and developmental traits such as stem elongation, branch number, flower or foliage color, fruit maturity, diseases, and content of phytochemicals by using supplementary UV radiation or UV-screening systems. (Oren-Shamir & Levi-Nissim, 1997; Bacci *et al.*, 1999; Paul & Gwynn-Jones, 2003). Although there is variation in the intensity of UV radiation in the different growing regions, spectral modification using different cladding material has a significant effect on the regulation of plant growth, morphology, and the cell composition in a range of plant species (Gautier *et al.*, 2005; Stamps, 2009).

1.6.1 Control of morphology

Control of morphology is important in commercial greenhouse production. High quality compact plants are easy to handle and transport, and more plants can be produced per unit area of greenhouse space. Regulation of stem length and plant shape without application of plant growth retardants is an important goal in an environmentally friendly horticultural greenhouse industry. Temperature has been one important tool, and the diurnal temperature alternations have strong effects on the morphology of many plants (Moe & Heins, 1989; Myster & Moe, 1995; Torre & Moe, 1998). Further, many plant species are sensitive to a short temperature drop during the 24 h daily growth cycle. Temperature drop has been used successfully to control stem elongation of ornamentals such as poinsettia in periods when the temperature outdoors is low enough to reduce the greenhouse temperature substantially. However, in warm periods and warm areas, the outdoor temperature is too high to create a steep temperature drop, and some plant species are not sensitive to temperature drop (Myster & Moe, 1995) .

Light climate such as irradiance, photoperiod, and light quality can also be used actively to control plant morphology. Artificial lighting systems such as light emitting diodes (LED), inter-lighting, and light spectrum filtering techniques such as colored covering materials are some of the techniques used to regulate the light climate in plant canopies (Mortensen & Strømme, 1987; Oyaert *et al.*, 1999; Li *et al.*, 2000). Recently, the use of LEDs with a high proportion of B light as supplementary lighting was shown to inhibit shoot elongation in roses and poinsettia compared to the traditional high-pressure sodium lamps (Islam *et al.*, 2012; Terfa *et al.*, 2013). Also, other studies have shown that plants treated with B light have a reduced plant height as compared with natural light (Mortensen & Strømme, 1987). Although reduction in shoot height, internode length, and leaf size under UV radiation have been observed in many different plant species (Kataria & Guruprasad, 2012; Terfa *et al.*, 2014; Zhang *et al.*, 2014), not much work has yet been done with UV-B as a tool to regulate morphology in commercial plant production. Torre *et al.*, (2012) reported that both UV-B and UV-A radiations are efficient as a tool to modulate plant morphology in vegetables, bedding, and pot plants. In a pot-rose study (Terfa *et al.*, 2014), 30–40% reduction in shoot height and leaf area were reported under UV-transmitting plastic films. Moreover, plant treated with UV-B combined with high day temperature and low night temperature were the shortest with the smallest leaf area and the lowest number of nodes as compared to plants not exposed to UV-B (Singh *et al.*, 2014). The reduction in plant height under UV-B is mainly a result of a reduction in internode

length rather than fewer internodes (Kakani *et al.*, 2003). Leaves are one of the most important morphological parameters that influence the shape and architecture of the plant canopy. Any change in the quantity and quality of UV may be an important factor regulating the growth and development of leaves (Dillenburg *et al.*, 1995). Various studies have shown that removing UV-B radiation generally increases leaf area compared to UV treated leaves (Nogués *et al.*, 1998; Zhao *et al.*, 2003; Terfa *et al.*, 2014),

1.6.2 Control of pest and diseases

In most greenhouse conditions, pesticides and different agrochemicals have been used for the eradication of plant pathogens. However, because of human health and environment-related issues, such chemicals are not currently recommended (Illing, 1997). Manipulation of environmental condition including day length and spectral quality of the light may provide an alternative strategy to protect plants from pests and diseases in greenhouse production systems (Raviv & Antignus, 2004; Suthaparan *et al.*, 2010). Application of UV-radiation in the greenhouse may be used to inhibit the germination and development of the fungal pathogen. Also, UV radiation may help pollinator insects to orient and locate flowers (Jones & Buchmann, 1974; Willocquet *et al.*, 1996; Suthaparan *et al.*, 2012).

The cladding material that blocks UV radiation affects the reproduction and direction of insects in greenhouse. Changing the light quality in the UV range of the spectrum mainly affects arthropod pests (Raviv & Antignus, 2004; Díaz & Fereres, 2007). Furthermore, several reports have indicated that UV-A radiation is a necessary stimulus for white flies, aphids, and thrips to differentiate between their host plant and the environment, so the lack of UV-A affects orientation and dispersal activities (Antignus *et al.*, 2001; Chyzik *et al.*, 2003; Lamnatou & Chemisana, 2013b).

Some studies have also revealed that UV-absorbing plastic film that blocks near-UV light radiation (300–400 nm) in greenhouse cultivation can be effective in preventing different types of pests from entering the greenhouse (Lamnatou & Chemisana, 2013a; Shimoda & Honda, 2013). However, care should be taken because reducing UV radiation appears to increase susceptibility to herbivores (Paul & Gwynn-Jones, 2003; Gols, 2014). The plants exposed to elevated UV-B levels have more tannins and lignin than the plants grown under low level of UV-B, and these secondary metabolites have an ecological importance in influencing

the palatability and digestibility of plants and affecting herbivore and other plant–animal relationship (Gehrke *et al.*, 1995; Rozema *et al.*, 1997).

1.7 Greenhouse crop production in Ethiopia

Agriculture is an important sector in Ethiopia as a main source of food for the population and serves as a main contributing sector in the national economy. Crop production occurs in rain-fed farming systems in most parts of the country, and this accounts for more than 95% of the land cultivated annually (Deressa, 2007). However, a wide range of both biotic and abiotic stresses affects crop productivity in the country. Greenhouse crop production systems in Ethiopia are a young agricultural industry, but they are rapidly growing. Among all crops produced in Ethiopia, including the flower industry, cut rose production is rapidly expanding as compared to other African countries (Getu, 2009).

Greenhouse production systems help growers to control the climate, diseases, and pests for improvement of yield and quality of a product as compared to open field production systems. The most common greenhouse type is a basic greenhouse with steel construction covered with plastic films (mainly polyethylene), with fixed or adjustable single roof vents or side screens.

As the horticulture industry is intensified and market competition is increasing, growers are becoming more and more dependent on agrochemicals as a relatively reliable method for the improvement of yield, regulation of plant growth, and control of plant pathogens. However, because of the perceived risks to humans and the environment, the use of some agrochemicals, including plant growth regulators, are not recommended in agricultural crop production system (Rajapakse & Kelly, 1992; Ecobichon, 2001; Hough, 2014). Therefore, application of different techniques such as the manipulation of light and its interaction with the background climate are important in Ethiopian conditions to gain knowledge on how to produce high quality products for export.

In Ethiopia, most of the ornamental crops and legume plants are grown under relatively warm and sunny climatic condition, where photoselective and shade nets are required to screen the light spectrum and decrease the light intensity. The use of such photoselective filters and shade materials in Ethiopia is a new technology; therefore, knowledge of the radiation transmittance characteristics of shade materials is important when assessing the potential

benefits of different materials. It is well known that small differences in solar transmittance can have a significant effect on crop growth (Cockshull *et al.*, 1992).

Ethiopia is located near the equator and about 50% of the total land is characterized as a mountainous region with elevation higher than 1500 meters above sea level (masl) (Zeleke, 2010). Since UV levels depend, among other factors, on the distance sunlight has to travel through the atmosphere, and thus the altitude, in such areas relatively high levels of UV-B can be found at ground levels (Sullivan *et al.*, 1992). Therefore, plants that naturally occur in such high UV-B radiation environments may have evolved specific adaptations that protect them from the deleterious effects of UV-B radiation (Rozema *et al.*, 1997). Few studies on cultural plants have been performed in areas with such high natural UV radiation.

2 Aims of the present study

The main objective of this study was to improve the understanding of the impact of UV radiation on plant growth and development and the role of the background climate. Also, the study aimed to shed light on the UV-B signaling in pea since information from other species than *A. thaliana* is limited. The experimental work was carried out both in controlled growth-chambers, with the use of UV-B lamps, and at field conditions using a plastic film to screen solar UV radiation as well as different shade materials. The specific objectives were as follows:

Paper I: UV-B inhibition of stem elongation and leaf expansion in pea is associated with altered GA metabolism in apical stem tissue and altered GA and IAA metabolism in young leaves.

Using pea as a model plant in this study, we aimed at evaluating the effect of UV-B on shoot elongation when provided separately or in combination with a diurnal temperature-drop treatment, to shed light on the involvement of hormone physiology in this respect.

Paper II: UV-B signaling in pea involves *LONG1* and *LIP1*, homologs of *Arabidopsis thaliana HY5* and *COP1*.

To extend the knowledge on UV-B–signaling to plants other than *A. thaliana*, we evaluated the involvement of the pea *HY5* and *COP1*-homologues *LONG* and *LIP1* in UV-B responses in pea focusing on UV-B induced DNA damage, UV-B–protecting flavonoids and shoot elongation. We also aimed to shed further light on the effect of GA in these UV-B responses.

Paper III: The impact of UV radiation at high altitudes close to the equator on morphology and productivity of pea (*Pisum sativum* cv. Cascadia) in different seasons.

Using an approach with UV-transmitting and UV-blocking films, the aim of this study was to evaluate the effect of UV in different seasons (dry and wet) on vegetative growth, flowering, and productivity of pea plants grown at two different high altitudes (1700 and 2800 masl) in Ethiopia.

Paper IV: Effect of UV radiation on the growth and postharvest characteristics of three pot-rose cultivars grown at different altitudes.

The aim of this study was to test the role of natural levels of UV radiation at different altitudes in Ethiopia in growth responses such as morphology and flowering, postharvest water usage, and shelf life of different cultivars of pot-roses. These pot-roses were grown under UV-transmitting and UV-blocking films at different altitudes.

Paper V: Growth and morphology of pea (*Pisum sativum* cv. Oregon sugar pod II) grown under different shading screens in Ethiopian climatic conditions.

The aim was to assess the plant growth and productivity of pea under three different coverings and to evaluate their potential under Ethiopian growing conditions.

3 Materials and methods

3.1 Plant materials

The experiments were carried out in the growth-chambers at Norwegian University of Life Sciences (NMBU, Norway) and in field conditions at Hawassa University (HU: Ethiopia) and Hagresalam (Ethiopia). For the experiments, which were conducted in Norway, (1) *Pisum sativum* L. (cv. Torsdag) as wild type (WT) and (2) four mutants (*long1*, *lip1 la cry-s* and *le*) with "Torsdag" background were used. Pea has previously been widely used as a model plant for scientific purpose to investigate its response to thermoperiodic stem elongation, diurnal temperature change, hormone regulation (Grindal *et al.*, 1998; Stavang *et al.*, 2005; Stavang *et al.*, 2007; Stavang *et al.*, 2009) and productivity. Also, many mutants are available to study hormonal and light signal transduction. The *long1* mutant is not able to deactivate the conversion of GA_1 to GA_8 by *PsGA2ox2* (Fig. 3.) (Weller *et al.*, 2009) and are included in Paper II to evaluate the role of LONG1 (the pea homolog of the *A. thaliana* HY5) in UV-B-signaling with respect to morphology, sensitivity to UV-B radiation and production of UV-B-protecting flavonoids. LIGHT INDEPENDENT PHOTOMORPHOGENESIS 1 (LIP1*)* accumulates a lower level of GA_1 because of an up-regulation of *GA2ox2* and *GA2ox1* relative to the WT (Weller *et al.*, 2009). The *lip1* mutant was also studied in Paper II to evaluate the role of LIP1 in UV-B-signaling . Furthermore, the *le* mutant mutated in the *GA3ox1* gene (Lester *et al.*, 1997) and the *la cry-s* GA singaling mutant, which behaves like beingGA saturated (Reid *et al.*, 1992), was used to evaluate role of GA levels and GA signaling in UV-B responses (Paper II).

In addition, for the pea experiments conducted in Ethiopia, two commercial pea cultivars were used: *Pisum sativum* L cv. Cascadia and *Pisum sativum* cv. Oregon sugar pod II. Pea is an annual plant in the legume family (*Fabaceae*) and is one major economically important pulse crop which is used as food for human consumption and as feed for animals. The pea pod has also become an important product for exportation for many different African countries including Ethiopia. Three different pot-rose cultivars (Rosa x hybrid "Cygein," "Tom-Tom," and "Snow white") were used as models for cut roses because they are fast growing.

3.2 UV–tubes

The spectral distribution of the UV tubes used in this study is shown in Fig. 5. Three UV-B fluorescent tubes in (paper I) and two or three UV-B tubes in (paper II) (UVB-313, Q-panel) were used in each UV-B treatments. A 0.13 mm thick cellulose diacetate film was used to screen wavelengths shorter than 290 nm (Fig. 5).The irradiance from the UV-B tube was measured on the top of the plants with a broadband UV-B sensor (SKU340, Skye Instruments). In Papers I based on a calibration factor from a spectroradiometer (Optronic OL-756, Optronic Laboratories, Oraldo, FL, USA), the absolute UV-B irradiation of 0.45 W m^{-2} was used as the set point. In paper II different levels ranging from 0.25 W m^{-2} was used. However, during the temperature drop, the UV-B level was reduced by approximately 25% because of the reduced efficiency of the lamps at low temperature. This reduction was measured 2 h after temperature reduction. In paper I the growth chambers had UV-B-non-reflecting walls, whereas in paper II the chamber walls were UV-B-reflecting.

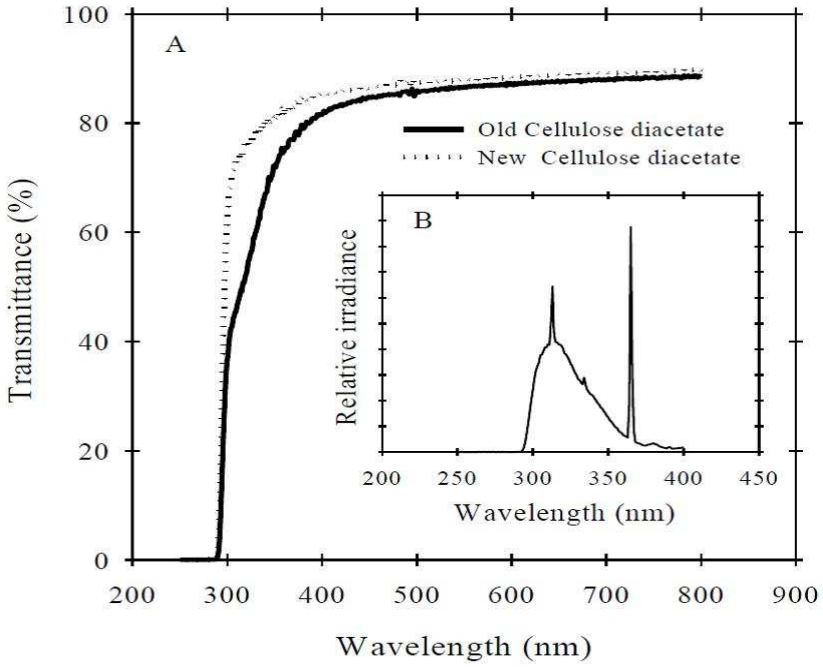

Fig. 5. UV-B spectrum transmittance (%) of new (dotted line) and old (dash line) cellulose diacetate foil (A) and UV-B tube (Q-panel UV313) relative irradiance under new cellulose diacetate (B) used in the growth-chamber experiment (2011–2014) at NMBU, Ås, Norway.

3.3 Real time PCR

In Paper I, real time RT-PCR with gene-specific primers and probes to monitor effects of UV-B and temperature-drop treatments on transcript levels of genes involved in GA and auxin-metabolism was analyzed. The methodology after Stavang *et al.*, (2005) was followed. The relative transcription level of 5 GA-biosynthesis genes (*IS*, *LH*, *NA*, *GA20ox1*, and *GA3ox1*) and two GA-deactivation genes (*GA2ox1* and *GA2ox2*) in pea and two IAA-biosynthesis genes (*YUC1* and *YUC2*) (papers I and II) were measured.

3.4 Field experiment

At field condition in Ethiopia, the impact of covering materials, altitude, and season on the growth and performance of commercially produced pea and pot-roses were evaluated (paper III, IV and V). The plants were grown at a high altitude (2800 masl) and a low altitude (1700 masl) under different plastic coverings transmitting UV-A and UV-B (+UV) or blocking UV-B and short UV-A (-UV). In the second experiment, we compared the impact of imported and locally produced covering materials on the growth and productivity of commercial pea cultivars *Pisum sativum* cv. Oregon sugar pod II (Paper V). In these studies, we evaluated the performance of cultivars in terms of growth morphology, stomata conductance, stomata morphology, dry matter accumulation, and pod productivity.

Fig. 6. Experimental layout established to evaluate the impact of UV-transmitting and UV-blocking films on the growth and productivity of commercial pea (*Pisum sativum* cv. Cascadia) at higher (2800 masl) and lower (1700 masl) altitude of southern parts of Ethiopia during the dry (January – April) and wet (April – June) season in 2012.

4 Main results and discussion

4.1 Effect of UV radiation on shoot elongation

It is well documented that shoot elongation is affected by different environmental factors including light quality, temperature, and UV-B radiation (Warrington *et al.*, 1976; Smith, 1982; Teramura & Sullivan, 1994; Stavang *et al.*, 2005). In this study, the effect of UV radiation on shoot elongation was investigated in different climatic regimes, in growth-chambers with the use of UV lamps, and in fields close to equator, having naturally high UV levels. In all experiments, UV radiation caused reduced shoot elongation (Papers I, II, III, and IV). Growth inhibition as a typical UV-B response is also reported in a wide range of other species such as petunia (*Petunia x hybrida*), cucumber (*Cucumis sativus*), red leaf lettuce (*Lactuca sativa*) rice (*Oryza sativa*), cotton (*Gossypium hirsutum* L.), mung bean (*Vigna radiata*), and sunflower (*Helianthus annuus*) (Finckh *et al.*, 1995; Ros & Tevini, 1995; Zhao *et al.*, 2003; Amudha *et al.*, 2005; Jayalakshmi *et al.*, 2011).

In the growth-chambers, plants were grown both at a constant temperature and with a temperature drop with or without UV-B radiation (Paper I and II). It was observed that a 6 h daily UV-B radiation combined with temperature-drop treatment from 21℃ to 13℃ (mean daily temperature of 20℃) inhibited stem elongation substantially by 30 % as compared to temperature drop only and 40% as compared to constant temperature (20℃) (Fig. 7). These results suggest that shoot reduction was stronger when plants were exposed to combined stresses (UV-B and temperature drop) compared to a single stress (UV-B alone or temperature drop alone). Similarly, in a study by (Ren *et al.*, 2007), a stronger synergetic effect of drought and UV-B radiation was found in the reduction of plant height, total leaf area, and specific leaf mass of *Populus kangdingensis* and *P.cathayana* species as compared to individual stresses.

In the field experiment performed in Ethiopia, the shoot reduction induced by UV in pea was almost similar, irrespective of the temperature (Paper III). The experiments in the field were performed with the use of UV-transmitting and UV-blocking films at a higher (1794–2800 masl) and lower (1700 masl) altitude. The results showed that, regardless of altitude and season, UV-B and some UV-A radiation from the solar spectrum reduced the shoot elongation of pea plants by about 15–19% as compared to the unfiltered solar spectrum (Paper III). On the other hand, in the experiment with roses, performed at the same field sites as the pea, the effect of UV radiation on shoot length was more prominent at the lower altitude (with higher

temperature). The reduction in shoot length was 10–15 % higher than at the higher altitude, despite the higher UV-B level at the higher altitude (with lower temperature) (Paper IV). In another study, the UV-B–induced reduction in the seedling growth of maize and sun flower was alleviated by a 4°C increase in temperature from 28°C to 32°C (Mark and Tevini, 1996). Thus, the interactive effect of temperature and UV on stem elongation probably varies with time, temperature range, and plant species.

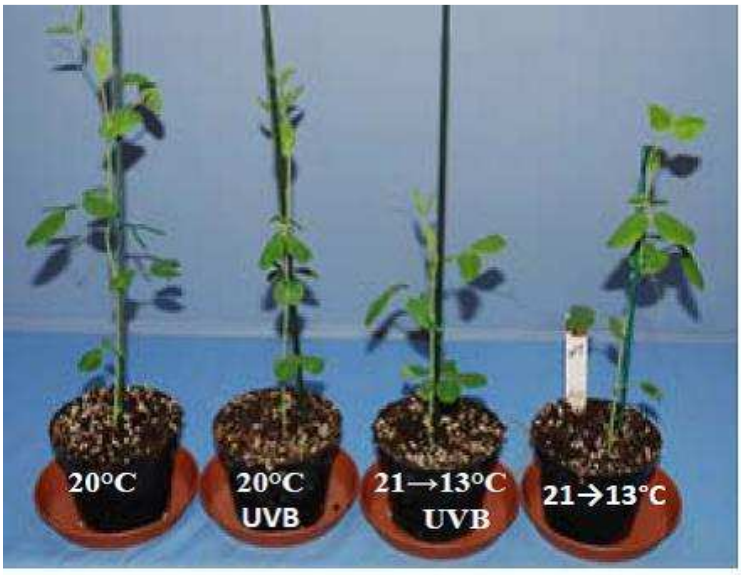

Fig. 7. Pea exposed daily to a UV-B radiation of 0.45 W m^{-2}, diurnal temperature drop (21℃ to 13℃), or the combination for 6 h in the middle of light period as compared to control. Daily average temperature was 20℃ in all cases.

4.2 Effects of UV-B radiation on other morphological changes

In addition to reduction in shoot elongation, the other morphological responses commonly seen in plants exposed to UV radiation are reduced apical dominance, increased auxiliary branching or tillering, reduced leaf area, change in SLA, and color changes (Jansen *et al.*, 1998). Pea plants exposed to UV radiation in the field in this study showed an increase in the number of branches (Paper III). Reduced apical dominance and stimulated branching is a characteristic growth pattern found in plants exposed to UV (Jansen, 2002). However, the plants exposed to

UV-B in the growth-chambers did not show any increase in the number of branches (Paper I). It could be that plants have to be exposed to UV-B for more hours per day to induce more branches or for a longer period of time. More branches were observed in poinsettia exposed to UV-B for 1.5 h during the night (Torre *et al.*, 2012). However, the poinsettia experiment run for several weeks. The pea plants in the chambers where only exposed to UV-B for ten days.

Leaves are photosynthetic organs, and thus, the leaf area and number of leaves are important in the growth and performance of plants. These parameters are commonly affected by various environmental signals. The growth-chamber experiments also clearly showed that UV-B exposure reduced the leaf area (Paper I). The reduction in leaf area is considered an adaptive strategy under non-optimal growth condition. The PAR light in the chambers was only 100 µmol m^{-2} s^{-1} which is very low compared to natural PAR levels. In the field experiments (Papers III and IV), the leaf area was not significantly affected by UV radiation but by altitude. As the altitude increased from 1700 to 2800 masl, the temperature dropped on an average by 0.7°C for every 100 m whereas the VPD decreased with the altitude. The lower leaf area (12–64%) and the lower numbers of leaves (21–44%) corresponded to higher temperatures and lower RH (higher VPD), especially in the dry season as compared to the wet season (Paper III). A low VPD commonly increases fresh weight and leaf area of various plant species (Mortensen, 2000). Thus, the strong decrease in the numbers of leaves and the total leaf area at the lower altitude during the dry season was probably related to a very high VPD.

4.3 UV-B–induced regulation of GA in apical stem and leaf tissue

In order to study the involvement of GA in response to UV-B radiation with respect to shoot elongation, GA_3 was applied to the apex or a fully developed leaf of the WT plant in a growth chamber experiment. Exogenous application of GA_3 counteracted the inhibitory effect of UV-B radiation on stem elongation, and this indicates a UV-B alteration of the GA metabolism (Paper I). It was also observed that the content of GA_1 was significantly reduced in the stem and the leaves under UV-B and UV-B combined with temperature drop (59% and 54% reduction in apical stem tissue and 69% and 64% in young leaves) (Paper I). However, earlier studies have demonstrated a reduction in the levels of bioactive GA in apical stem tissue in response to temperature-drop treatment or lower day than night temperature, including in pea (Grindal *et al.*, 1998; Stavang *et al.*, 2005; Stavang *et al.*, 2007), we could not detect a

significant effect of temperature drop only on GA_1 level in apical stem tissue. Since there is a gradient of bioactive GA_1 in shoot apices and the highest level is found in the part of the subapical meristem showing the largest degree of cell division and cell extension (Olsen *et al.*, 1995), it cannot be excluded that differences in harvested tissue in the previous and present studies might be a reason for the lack of effect of temperature drop on GA_1 level in the present study. In the previous study of pea where GA was quantified under temperature drop, elongating petioles were also analyzed together with the shoot tips (Stavang et al. 2007).

The precursor of the bioactive GA and its inactivation under the UV-B and temperature combination was studied. The results indicated that the level of GA_{44}, GA_{19}, and GA_{20} and the first inactivation product GA_8 were reduced in the apical stem in response to a daily UV-B treatment, irrespective of the temperature (Paper I). In the leaves there was a 55% and 40% reduction in GA_8 under a constant temperature and a temperature drop, respectively, in response to UV-B. These observations might suggest that GA biosynthesis as well as GA inactivation is affected by UV-B . This was confirmed by that the ratios of the GA inactivation products GA_8 and GA_{29} to their precursors, GA_1 and GA_{20}, respectively, showed an increasing trend in the stem and the leaves in response to UV-B (Paper I).

In pea, the largest effect of temperature drop on GA level in regulation of shoot length was through modulation of the level of *GA2ox2* and *GA2ox1 (Stavang et al., 2007; Olsen et al., 2009).* Both an increase in the level of GA_8 and GA_{29} might have been expected to have a higher expression of *GA2ox1* and *GA2ox2* under UV-B treatment in the stem and the young leaves (Paper I), but this was not the case for the apical stem. Although not statistically significant at $p \leq .05$, the trends of increased transcript levels of the GA inactivation genes *GA2ox2* and *GA2ox1* support increased GA inactivation in response to UV-B in leaves (Paper I). Like several GA metabolism genes expression of *GA2ox1* and *GA2ox2* is known to exhibit a diurnal variation s, Stavang *et al.* (2005), and it might thus well be that sampling at other time points during the diurnal cycle would have shown a signficant effect on the transcript levels of these genes.

However, exposure to a temperature drop significantly increased only the transcript level of *GA2ox2* by about 3-fold in apical stem tissue (3 h into the temperature-drop treatment) as compared to constant temperature at the same daily mean temperature. Such an increase is consistent with earlier studies of pea exposed to a temperature drop or lower day than night temperature (Stavang *et al.*, 2005; Stavang *et al.*, 2007). Collectively, these results and the increased ratios of the GA inactivation products GA_8 and GA_{29} to their precursors GA_1 (bioactive) and GA_{20} (precursor of GA_1), respectively, in the apical stem tissue in the present

study might imply a general role of GA inactivation in adjusting growth under conditions unfavorable for extensive shoot elongation.

However, in line with the current view on UV-B, such adjustment of GA levels, and accordingly, reduced shoot elongation in response to ambient UV-B levels can be considered as part of the adaptive behavior of plants to the environment (Hectors *et al.*, 2007). Thus, the trends of increase in transcript levels of *GA2ox1* as well as *GA2ox2* in response to daily UV-B exposure is consistent with the increased ratios of GA_{29} to GA_{20} and GA_8 to GA_1. This is also consistent with the previously demonstrated increase in transcript levels of *GA2ox1* in leaves of *A. thaliana* exposed to UV-B (Hayes *et al.*, 2014b) (Fig 4).

4.4 Effects of UV-B on IAA and ABA content in apical stem and leaf tissue

To further understand the impact of the combined effect of UV-B radiation and temperature on IAA, we analyzed the content of IAA and IAA conjugates in the stem and leaf of the pea plant (WT). However, no effect of UV-B on IAA levels or IAA conjugates (IAA-Asp and IAA-Glu) in the apical stem tissue was observed in the pea plants from the two temperature regimes (Paper I).

In contrast to the effect on the apical stem tissue, the level of IAA decreased in the young leaves of pea in response to the daily UV-B exposure (significant at $p \leq 0.05$ under constant temperature, and showed a trend of decrease under temperature drop (Paper I). The significant effect of UV-B on the ratio of the IAA conjugates recorded (IAA-Asp and IAA-Glu) to IAA in the young leaves in the present study (paper I) indicates that the reduced IAA levels are at least partly due to enhanced conjugation although an effect of other IAA biosynthesis cannot be excluded.

Furthermore, the transcript levels of *YUC1* and *YUC2* were not significantly affected by UV-B or temperature-drop exposure, except a possible slight trend of reduced *YUC1* transcript level under UV-B (paper I). It should be noted that although *YUC* genes have been suggested to be involved in IAA biosynthesis and are affected by light quality and temperature, their role as rate-limiting in IAA biosynthesis is currently debatable (Tao *et al.*, 2008; Stavang *et al.*, 2009; Ross *et al.*, 2011; Tivendale *et al.*, 2014).

The endogenous ABA has many roles in the growth and development of plants. The results indicated that the content of ABA and the inactivation products of DPA and neo-PA in

the apical stem tissue were significantly lower in the UV-B–treated plants as compared to the control plants (Paper I). In young leaves, there were also trends of decrease in ABA in response to UV-B, and the inactivation product DPA was significantly reduced (Paper I). Such a trend of an UV-B–induced decrease in ABA differs from the earlier published results from the leaves of species such as maize and grape wine, where the increase in ABA in response to UV-B was shown to stimulate production of UV-B–protecting compounds (Berli *et al.*, 2010; Tossi *et al.*, 2014). The reason for this difference remains elusive, but nevertheless, the levels of certain flavonoids known to protect against UV-B increased in response to UV-B also in the pea plants of the current study (Paper II).

4.5 Genotype sensitivity to UV–B radiation and UV-B signaling

Plant sensitivity to UV-B can be explained either in terms of the visual damage or by a number of changes in agronomic characteristics such as plant height, leaf area, and dry matter accumulation. However, response to UV-B varies from species to species. Plants grown under enhanced UV-B radiation showed unusual growth patterns and developed different visible stress symptoms including formation of necrotic spots and color on the leaves or the stem (Caasi-Lit *et al.*, 1997) or enhanced the accumulation of UV-B–absorbing substances (Smith *et al.*, 2000). Ultraviolet-B radiation has a significant inhibitory effect on the growth and biological yield of several crops (ELV & JMG, 1998; Krizek *et al.*, 1998).

In this study, aiming at shedding light on the UV-B signaling in pea, we evaluated the sensitivity of WT, *long1, lip1, le, and la cry-s* to UV-B radiation under a constant temperature and a temperature drop in terms of shoot elongation, DNA damage and level of UV-B absorbing substances. In *A. thaliana* HY5 and COP1 are known to be important players in UV-B signaling, enhancing formation of UV-B protecting compounds and resulting in reduced shoot elongation and decreased leaf area. We hypothesized that in pea the HY5 and COP1 homologs, denoted LONG1 and LIP1 (Weller *et al.*, 2009), respectively, play similar roles.

In this study (paper II) it was observed that when the WT showed a certain degree of visual damage such as leaf edge curling in response to a daily 6 h UV-B radiation under constant temperature for 10 days, the *lip1, le* and *la cry-s* mutants showed no or less such damage. In contrast, a daily 30-minute UV-B radiation under constant temperature resulted in more leaf curling in *long1* than WT (paper II). This suggests that the *lip1, le* and *la cry-s* were

stronger in resisting deleterious effect of UV-B radiation as compared to WT, whereas *long1* was the most sensitive to UV-B radiation. Leaf curling is a photomorphogenic response that might be used by the plant to reduce the leaf area exposed to the UV-B radiation (Greenberg *et al.*, 1997; Jansen *et al.*, 1998). Leaf curling and twisting of shoot tips in *long1* genotype is in accordance with previous observations of the UV-B-hypersensitive *hy5* mutant in *A. thaliana* (Brown *et al.*, 2005; Gerhardt *et al.*, 2005; Jenkins, 2009). It is well known that plants lacking UV-B–protective compounds are hypersensitive to UV-B radiation and oxidative damage, and this was shown to be the case for *hy5* mutant (Landry *et al.*, 1995).

4.6 UV-B signaling related to effect of UV–B radiation on DNA damage

It is well known that high levels of UV-B radiation induces DNA damage in plants (Hollosy, 2002). The most common DNA photoproducts are cyclobutane-type pyrimidine dimers (CPD) and the pyrimidine (6,4) pyrimidine dimer(6,4-PPs) (Lo *et al.*, 2005). In our study, we evaluated UV-B–induced DNA damage in different pea genotypes by measuring the level of CPD which was commonly reported as the highest proportion of UV-B–induced DNA damage (Britt, 1995; Hollosy, 2002). Under a 6 h UV-B irradiation, the WT grown under constant temperature had higher CPD than the *lip1, le* and *la cry* mutants (Paper II). Thus, in line with their lower degree of visible UV-B-related damage, the *lip1*, *le* and the *la cry-s* mutant both showed lower CPD levels than the WT. This indicates enhanced protection mechanisms towards UV-B-related damage in these mutants compared to the WT. Interestingly, higher UV-B resistance in the *lip1* mutant is actually opposite to the situation shown for the *cop1-4* mutant, which is more sensitive to UV-B related damage than the WT (Oravecz *et al.*, 2006). On the contrary, the *long1* mutant had higher CPD content than the WT. This demonstrates that the *long1* mutant, like the *hy5* mutant in *A. thaliana*, has a less developed UV-B protective mechanism (Brown *et al.*, 2005; Gerhardt *et al.*, 2005; Jenkins, 2009).

4.7 UV-B-signaling related to effect of UV-B on shoot elongation

In pea, the light induced interaction of LIP1 (LIGHT-INDPENDENT PHOTOMORPHOGENESIS 1) and LONG, the pea ortholog of *A. thaliana* COP1 and HY5, respectively, are important to regulate the expression of the GA catabolism gene *GA2ox2* and

the levels of the active GA, GA_1 (Weller *et al.*, 2009; Li & Huang, 2011). It was demonstrated that light reduced the length of etiolated pea plants and the content of bioactive GA_1 in the WT but in the *long1* mutant light didn't affect GA levels (Weller *et al.*, 2009).

In our study, it was observed that a daily 6 h UV-B radiation, either provided alone or in combination with temperature drop significantly reduced shoot elongation in WT and the *lip1* mutant. Thus, LIP1 is apparently not involved in UV-B-signaling resulting in reduced elongation growth. The *long1* mutant did not respond to UV-B at all, and also not to temperature drop exposure. This demonstrates that LONG1 is an important signaling component involved in UV-B-inhibition of shoot elongation. The lack of response is probably associated with lack of ability of the *long1* mutant to down-regulate its levels of bioactive GA_1 under UV-B like upon transfer of etiolated seedlings to light (Weller et al., 2009). Such a situation has also previously been observed under temperature drop and lower day than night temperature (Wendell *et al*., unpublished, PhD thesis Wendell, NMBU 2013). The *le* mutant did not respond to UV-B under constant temperature, but showed a slight response of reduced shoot elongation under the combined treatment. The *la cry-s* mutant did not respond neither to UV-B or temperature drop treatment and was elongated independently of the environmental conditions provided (paper II). These observations support that regulation of the GA levels or the GA response (signaling) is required for UV-B-mediated down-regulation of shoot elongation.

UV-B-related reduction of shoot reduction appeared stronger (WT= 46%; *lip1*= 51% and *le* = 20%) under the combined treatments (6 h daily UV-B radiation and temperature drop in the middle of the light period) as compared to control plants (paper II). Under a short UV-B irradiation (30 min), shoot reduction in WT was reduced under the combined treatment (UV-B and temperature drop) in contrast to plants grown under constant temperature. The higher shoot reduction under the combined treatment might be related to that the UV-B-temperature-drop conditions together are perceived as more stressful than either condition alone.

UV-B–induced shoot length reduction in WT and *lip1* as well as the slight response of *le* under UV-B-temperature drop exposure, might be related to reduction in the content of GA_1 and IAA (Paper I). Moreover, tissue damage resulting from high levels of UV-B radiation results from inhibition of the photosynthetic process, degradation of proteins and DNA, and an increase in oxidative stress that leads to lower performance of plants (Stapleton, 1992; Strid *et al.*, 1994). A reduction in the biomass accumulation is the cumulative effect of tissue damage

or inhibited physiological function; therefore, a lower biomass accumulation is a reliable indication of a plant's sensitivity to UV-B radiation (Smith *et al.*, 2000). In this study, we observed that genotypes treated with UV-B treatments had a lower accumulation of dry matter but with a higher level of total phenolic compound, except in *long1* genotype (data not presented). This might be related to an adaptive strategy to allocate more metabolic output for production of phytochemicals for defense rather than growth. A report indicated that plants have the ability to balance efficient substrate use for different physiological processes and developmental states based on the environmental growth conditions (Thornley & Cannell, 2000).

4.8 UV-B signaling related to effect of UV–B radiation on levels of phenolic compounds

Plants exposed to UV radiation accumulate different secondary metabolites as a protective mechanism to absorb UV radiation and prevent cellular damage from incoming UV radiation (Jansen *et al.*, 2008; Zhang & Björn, 2009). Plants with a higher concentration of flavonoids are less sensitive to UV-B radiation than genotypes mutated in their flavonoid biosynthesis (Landry *et al.*, 1995). Such differences in flavonoids and other phenolic compounds between UV-B sensitive and the UV-B tolerant genotypes have been reported by a number of investigators (Ormrod *et al.*, 1995; Caasi-Lit *et al.*, 1997). The accumulation of phenolic compounds in the epidermal layers of leaves is thus an important mechanism to avoid the damaging effect of high levels of UV-B radiation.

In our study, eighteen different phenolic compounds were detected in pea leaves by HPLC analysis (paper II). However, in our discussion we focused on three major flavonol glycosides, quercetin, kaempferol and myricetin as well as two major flavones, luteolin and apigenin. Following 10 days of 6 h daily UV-B irradiation, the *lip1* and *le* mutants had higher levels of quercetin, kaempferol, and apigenin glycosides and total phenolic compounds than the WT. Thus, the lower levels of damage and CPD levels in the *lip1* and *le* mutant than the WT, can probably be ascribed to the higher levels of these flavonoids in these mutants. On the other hand, *long1* had lower accumulation of these UV-screening substances than the WT in response to 30 min daily UV-B exposure (paper II). Accordingly, the more visual damage/leaf curling

and DNA damage in *long1* than the WT might be thus well be related to the lower content of these UV-screening substances (paper II). This is, consistent with several reports demonstrating that plants lacking flavonoid compounds are more sensitive to UV-B radiation than the wild type (Li *et al.*, 1993); Jenkins, 2014).

4.9 Stomata conductance and SLA depends largely on the background climatic conditions such as temperature and VPD and less on UV radiation

Stomata conductance has been shown to increases with increasing altitude (KoÈrner & Cochrane, 1985). However, stomata responses to UV radiation in different plants vary based on the background climatic factors and the origin of the plants. Plants originating from a higher altitude or a higher UV-B regions are less sensitive to enhanced UV-B than those from a low UV-B location (Chalker-Scott & Scott, 2004). The results from the higher altitude indicated that the pea plant exposed to UV radiation had higher stomatal conductance than the plants grown without UV exposure (Paper III), whereas the UV radiation did not affect stomata conductance in all pot-rose cultivars which originated from higher altitude (Paper IV). The fact that UV radiation increased stomatal opening at a higher altitude but not at a lower altitude indicates interplay with other climatic factors. Moreover, the effect of UV-B on stomata behavior is dependent on the UV-B fluence rate. In general, a very low UV-B fluence rate stimulates the stomatal opening whereas a higher dose induces closure (Nogués *et al.*, 1999; Jansen & Van Den Noort, 2000; Eisinger *et al.*, 2003; He *et al.*, 2005; He *et al.*, 2013). However, the different stomatal response to UV in the present study is rather an effect of the background climate than the UV-B dose. Increasing stomata conductance, stomata frequency, and leaf thickness are found in many plant species with increasing elevation (Körner *et al.*, 1986). Such changes in leaf characteristics with the altitude might be because of fluctuations in temperature and the amount of light intercepted by the leaf.

Plants can adapt to their light environment through modulation in the biomass distribution to the different parts of the plant or through changing the plant anatomy including leaf area and specific leaf area (Evans & Poorter, 2001). A given amount of biomass can be spread over a large or small area. The SLA is leaf area per unit leaf biomass. Plants grown under high light intensity generally have thicker leaves with a lower SLA (Poorter & Van der Werf, 1998). A higher PAR in the dry season generally (except for at +UV at highest altitude) correlated with a decreased SLA (Paper III). In our study also, the significant difference in the

irradiance levels (PAR) in the different seasons and altitudes had probably affected the SLA more than the effect of UV-B radiation. This corresponds with the investigation of (Meziane & Shipley, 1999) in which a strong negative correlation was observed between the SLA of different herbaceous plants and the levels of irradiance. Under natural growing conditions with UV present, various reports have shown that the SLA varies with the leaf age (Reich *et al.*, 1992; Coleman *et al.*, 1994; Reich *et al.*, 1999), altitude, and length of the growing seasons (Körner, 2007). A report from (Moser *et al.*, 2007) indicated that the average SLA was significantly different at different altitudes, with up to 40% higher SLA at the lowest altitude (1050 masl) as compared to highest altitude (2380 masl).

4.10 Chlorophyll fluorescence

Chlorophyll fluorescence has been shown to be a useful tool in the detection of environmental stress such as UV and light-induced photoinhibition (Krause & Weis, 1984; Larkum & Wood, 1993). In the growth-chamber experiment (Paper I), no significant difference was found in maximal photosystem II efficiency (Fv/Fm) between plants from different temperature regimes (constant temperature and temperature drop) or plants with or without UV-B exposure (results not shown). However, in the field experiments (Papers III, IV, and V), the Fv/Fm was significantly different between treatments for pea but not roses. The Fv/Fm value measured in roses was similar irrespective of the UV exposure and the value was within the range common for healthy sun-adapted plants at both the higher and lower altitudes (0.8±0.05) (Schiefthaler *et al.*, 1999). On the other hand, the Fv/Fm value measured in the pea plants was lower in the dry season at the higher altitude. The lowest Fv/Fm (highest stress, Fv/Fm= 0.66) was found with solar UV present at the higher altitude during the dry season (Paper III). The significant decrease in the pea plant height at the higher altitude during the dry season might be because of the higher UV and/or PAR levels caused photoinhibition which reduced the Fv/Fm (Paper III). Moreover, other reports have indicated a negative correlation between the irradiance and the Fv/Fm ratio in plant species grown in field (Dawson & Dennison, 1996). Thus, not only UV-B but probably also the combined effect of high levels of PAR and high air temperature in the dry season reduced the Fv/Fm value of the pea plant at the higher altitude. However, there was no clear relationship between the number of pods and the value of Fv/Fm.

4.11 UV radiation affects time of flowering in pea and roses but has no effect on pea pod production

It is well known that flowering time is affected by environmental factors including temperature, photoperiod, and light quality. Controlling the time of transition from vegetative growth phase to reproductive growth stage and synchronizing flowering with environmental factors is important for successful agriculture and horticulture crop production. In this study, the combined effect of the altitude, UV exposure, and season on plant productivity and days to appearance of the first flower bud were observed using pea and pot-roses as model plants (Papers III and IV). Although flowering time in most plant species varies with genetic as well as environmental factors, flowering in pea has been reported to commonly start about 40–50 days after planting in the field (McKay *et al.*, 2003). However, in these studies it was found that the flowering time for pea and pot-roses grown under UV-transmitting film was significantly delayed by 2.5 to 4.8 days and 7 to 10 days, respectively. Both species had the earliest flowering when UV-B and the shortest wavelengths regions of UV-A were excluded from the solar spectrum.

UV-B–induced delay in flowering has also been reported in other species such as maize (*Zea mays*) and petunia (Staxén & Bornman, 1994; Saile-Mark *et al.*, 1996; Caldwell *et al.*, 1998; Terfa *et al.*, 2014). In roses, it was suggested that the delay in flowering might be an indirect effect of UV radiation because of the reduced leaf area, resulting in lower light capturing and lower dry matter accumulation. Sugars are important both as specific signals for the activation of some genes and as energy source for carbon metabolism in the development of flowers (Schiefthaler *et al.*, 1999). In pea, no difference in the total leaf area was found between +UV and -UV as in the roses; thus, the delayed flowering under +UV might be stress related. Although flowering time was affected in our study, the number of pods was not affected much by the UV-radiation. Rather, the results of our study revealed that the number of pods per plant at the end of the experiments was strongly affected by the number of leaves and the SLA.

4.12 Effect of UV–B on postharvest performance of pot–roses

In this study, the postharvest life of three rose cultivars was tested and found to be significantly affected by the altitude and not by the UV radiation (Paper IV). Leaf wilting and leaf drying

are typical postharvest characteristics for water-stressed plants (Torre & Fjeld, 2001). Hence, plants grown at the lower altitude showed a higher percentage of leaf drying and wilting when moved to a test room. Further, the postharvest transpiration was also found to be higher in plants developed at lower altitude as compared to those developed at a higher altitude. They had twice as high water usage as the high altitude plants when transferred to the postharvest room (VPD of 1.2 KPa). It has been questioned for many years whether the UV radiation has a positive influence on postharvest life of roses. This study shows that the UV radiation has no effect on postharvest performance of pot-roses and probably not on cut roses. The main reason for a shorter postharvest life of cut roses is connected to stomata function and water usage and UV radiation did not have any effect on these parameters.

4.13 Effect of three different covering materials – the cheap locally produced can be used for pea production

Controlled plant production systems offer the possibility of providing high quality crops with higher productivity. High quality and higher productivity of horticultural crops can be achieved within efficient, cost effective, and well-structured greenhouses (Giacomelli & Roberts, 1993). Many reports have indicated that the selection of the covering material has significant influence on the crop quality and productivity (Shahak *et al.*, 2004a; Shahak *et al.*, 2004b; Espi *et al.*, 2006).

In this study (Paper V), three different covering materials were used to evaluate their effects on the growth and development of peas in the Ethiopian climate. The microclimate measured inside the “greenhouses” did not show significant differences in temperature or air humidity but the light climate was different (Paper IV). The total PAR transmitted through the Svensson covering material was 50% less than the PAR transmitted through the two other plastic films. The reduction in PAR under the Svensson screen material might be related to the effect of dust and the amount of light diffused through the covering material. A larger tent size probably gives more diffused light than a smaller tent size.

Moreover, colored shade nets have a tendency to increase light scattering, depending on the concentration of the dye and the design of the net, and an increase in light diffusion may influence plant development and growth (Fallik *et al.*, 2008; Shahak *et al.*, 2008). The changes in the PAR that we observed between the white and yellow plastic films can be explained by the dye intensity and light-scattering nature of the film. As it was reported by (Tatineni *et al.*,

2000) that changing the dye concentration of the plastic film had a major role in changing the light spectrum beneath the plastic films. Light diffusion is important in a greenhouse production system as it improves the overall light distribution throughout the plant canopy. Previous reports have confirmed that the plants grown under diffused light intercept more light than the plants grown under direct light, and the roof material with diffused light results in a lower leaf temperature and the optimal photosynthesis, thus increasing the final yield (Pollet *et al.*, 2000; Hemming *et al.*, 2005; Hemming *et al.*, 2007).

4.14 Effect of covering material on stomata conductance, Fv/Fm, and pod production in pea

Environmental stress including light radiation, temperature and UV-B radiation has a significant effect on plant growth and developments. Removal of UV-B from the growth environment has been a common strategy to avoid UV-related stress in plants. However, the stress response to UV-B radiation can be crop or dose specific (Allen *et al.*, 1999; Randriamanana *et al.*, 2015). Maximal PSII efficiency (Fv/Fm) measurements are used as diagnostic tools that help in assessing the plant damage caused by environmental stresses such as high PAR, UV-B radiation, and drought (Roháček *et al.*, 2008). Fv/Fm ratios near 0.83 indicate unstressed plants (Bongi & Loreto, 1989; Duke *et al.*, 2001). In our experiment, the lowest Fv/Fm (0.77) value was recorded on pea plants grown under the locally produced yellow covering material. This may be related to the higher level of PAR and UV radiation received under yellow plastic film than the other two imported covering materials. It is well known from other studies that high light intensity may result in an energy imbalance that often leads to photo inhibition or inactivation of PSII (Apel & Hirt, 2004; Chaves *et al.*, 2008). A higher Fv/Fm (0.83) value was measured from those plants grown under the Svensson film as compared to the white and yellow plastic films.

Further, the stomata aperture, stomata area, stomata conductance, and transpiration rate of pea plants was reduced more under the yellow covering material than under the two the imported covering materials. The lowest Fv/Fm always corresponds to the lower stomata conductance (Prieto *et al.*, 2009) and this report coincides with our investigation under the yellow plastic film. However, there was no significant difference between the Svensson and white plastic films (Paper V). The reduction in stomatal aperture and conductance helps to

control the transpirational water loss and give an optimal plant growth without affecting productivity.

Therefore, any agronomical techniques used to minimize water loss through modulation of plant morphology and physiology may allow farmers to produce crops such as pea under yellow plastic film with optimum productivity under a water stress condition. Modification of growth parameters such as plant size, leaf area, and stomata conductance using climatic factors for control might be a good method to optimize water use efficiency.

5 Conclusions and future prospective

5.1 Conclusions

- UV-B radiation has a stronger reducing effect on shoot length and leaf area of pea plants when provided together with a daily temperature-drop treatment as compared to a constant temperature
- This inhibition of shoot elongation and leaf expansion in pea is associated with the modulation of the GA metabolism in the shoot apices and altered metabolism of GA and IAA in young leaves. Ability to adjust the GA levels or GA response was shown to be required for the UV-B induced reduction of shoot elongation.
- Reduced level of the bioactive GA_1 in response to UV-B is apparently due to increased GA inactivation in both tissues and probably decreased biosynthesis, at least in the leaves. Reduced level of IAA in leaves appears to be associated with an increased IAA-conjugation.
- Like HY5 in *A. thaliana*, LONG1 is an important UV-B signaling component in pea with respect to flavonoid production, protection towards UV-B-related damage, and inhibition of shoot elongation, as judged from the hypersensitivity of the *long1* mutant to DNA-damage, low levels of specific flavonoids and no effect of UV-B on shoot elongation.
- Mutation in LIP1 makes the plants more UV-B resistant with higher flavonoid levels. In these respects LIP1 does accordingly seem to act opposite to *A. thaliana* COP1 in UV-B signaling. The similar UV-B-induced inhibition of shoot elongation in the *lip1* mutant and the WT indicates that LIP1 is not involved in signaling in this respect.

- The GA deficit *le* mutant and the GA signaling mutant *la cry-s* were less sensitive to UV-B-related damage compared to WT, probably due to higher flavonoid levels, as shown in *le*. Thus, GA levels or GA-response do not affect susceptibility to UV-B-related damage.
- UV radiation either at a higher or lower altitude inhibited elongation growth and delayed the flowering both in pea and rose cultivars but had no significant effect on pea productivity or postharvest behavior of roses. Thus, other climatic factors (PAR, temperature, and VPD) have a stronger effect than UV radiation.
- Pea is robust to light quality and the three different screening materials tested in this study did not result in differences in yield. Thus, the cheap locally produced screening materials is of current interest as an alternative for pea production in the Ethiopian climate.

5.2 Further perspective

Exploring the use of UV radiation for horticultural purposes is of future interest. To manipulate UV radiation in natural light is relatively easy with the use of screening materials and in controlled environment by adding artificial UV. It can be used both in the field and inside greenhouses. The UV lamp technology (UV-LEDs) is also emerging and is expected to be more efficient and less expensive within the next 10 years.

In many different countries, the use of plant growth retardants will disappear in a few years, and the growers will need alternative strategies to control elongation growth. The use of temperature as a tool is a relevant method in Scandinavia. However, in periods when the temperature outside is too high, it is difficult to obtain an effective temperature drop inside the greenhouse. In these periods, UV exposure can be used to induce growth inhibition, maybe together with temperature manipulation. The work in this thesis clearly shows that the combined effect of temperature drop and UV-B exposure is an efficient tool to control shoot elongation. However, to use this method in commercial greenhouses, great care must be taken to avoid UV-related damage not only to plants but also to the workers in the greenhouse. More knowledge on the effects of artificial UV on insects is also needed because biological control is commonly used in greenhouse systems of today. Night-time exposure could be an alternative to avoid problems for the workers in the greenhouse. This has been tested by (Suthaparan *et al.*, 2013; Suthaparan *et al.*, 2014) and shows that night-time exposure is effective, but plants are more sensitive as compared to daytime. The work of (Suthaparan *et al.*, 2013; Suthaparan *et al.*, 2014) also shows that the short wavelength of UV-B is efficient in controlling powdery mildew in roses, cucumber and strawberry. To combine disease control and stem elongation control is an interesting thought. Since the effect of UV also varies with the background climate, as shown in Papers I–IV, further studies are required to understand the interaction between the UV-B radiation and the other climatic factors on different plant species.

It is well known that enhanced UV-B radiation affects the biomass and content of secondary metabolites in plant tissue. In Paper II we demonstrated that 6 h daily application of UV-B radiation-induced the accumulation of phytochemicals including glycoside forms of flavonols, quercetin, kaempferol, and myricetin as well as major flavones, luteolin and apigenin in pea leaves. Information from this study can be useful for the regulation of secondary metabolites production in plant products with superior quality under controlled environmental

condition. However, further study is required to balance the metabolic cost used in biomass accumulation and the biosynthesis of secondary metabolites.

Pea pods are an important product for export to Europe. To grow the pea pods in the highlands with naturally high levels of UV might be an important future venture. However, there is a need to document the quality and the levels of secondary metabolites in pods from the highlands. Further, UV radiation also has a strong effect on the development of plant pigments including anthocyanin, carotenoid, and chlorophyll in fruits, flowers, and leaves. Moreover, it has been tested as a postharvest treatment to improve the color and nutritional benefits of different fruits and vegetables. However, further study is required to determine whether the dosage of UV-B radiation, time of exposure, cultivar, and storage climate have an impact on the physiology and nutritional composition of stored horticultural products. UV-C can also be an alternative in postharvest treatments. In Ethiopia, this can be a useful method to control postharvest diseases as this is a huge problem for many tubers, vegetable, and fruits. Exposure prior to storage or during storage must be tested. Further, to gain a deeper knowledge into this area further studies are required to evaluate plant pathogen–host interaction, sensitivity of plant genotypes, and physiological changes under enhanced UV-B radiation.

Moreover, the experiments in this thesis revealed that stomatal responses to UV varied with the background climate. Especially, it seems to be an interaction between UV and VPD. More knowledge on the regulation of UV on stomatal behavior is required. In Ethiopia, shortage of water is a main problem in addition to excessive light (and high temperatures). To find screening materials that reduce water consumption but at the same time give a high yield is extremely important for the future food production in Ethiopia.

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Paper I

Amsalu Gobena Roro, K. A. Solhaug, Tone Ingeborg Melby, S. Torre, and J. E. Olsen

UV-B-inhibition of stem elongation and leaf expansion in pea is associated with altered GA metabolism in apical stem tissue and altered GA and IAA metabolism in young leaves

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Summary

UV-B radiation typically reduces plant size and leaf area. In *Arabidopsis thaliana* this is associated with reduced indole-3-acetic acid (IAA) levels and, although GA levels have not been reported, there are indications of altered gibberellin (GA) metabolism. However, information on the impact of UV-B on hormones controlling different aspects of plant morphology including stem elongation in other plant species is limited. The aim of this study was to assess the effect of non-damaging levels of UV-B on metabolism of hormones controlling stem elongation and leaf expansion in pea (*Pisum sativum*). Six hour daily UV-B exposure (non-damaging level) for 10 days reduced shoot elongation by 9% and 30%, respectively, when provided under constant temperature (20ºC) and together with a daily temperature drop (21ºC to 13ºC; daily mean temperature 20ºC), a regime

commonly used to control shoot elongation in temperate-zone greenhouse industry. Thus, the results indicate a stronger effect of UV-B under lowered temperature and such treatment is thus potentially an efficient elongation-controlling tool. UV-B reduced leaf area by 35% and 18% in the two temperature regimes respectively. These morphological changes were associated with reduced levels of bioactive gibberellin GA_1 (by 54-69%) in apical stem tissue and young leaves, apparently due to increased GA inactivation, and possibly reduced GA biosynthesis, at least in leaves. Consistent with this, exogenous GA_3 counteracted UV-B-induced inhibition of shoot elongation. UV-B reduced the IAA levels in young leaves only (27-35%) under both temperature regimes, apparently through increased conjugation. Furthermore, ABA and some ABA metabolites decreased in response to UV-B but the significance is unclear. In conclusion, UV-B-induced inhibition of shoot elongation and leaf expansion in pea is associated with modulation of GA metabolism in shoot apices and altered metabolism of GA and IAA in young leaves.

***Key words**:* Abscisic acid, auxin, gibberellin, *Pisum sativum*, temperature drop, UV-B

Introduction

The ultraviolet (UV) radiation of the solar radiation has three different regions: UV-C (220-280 nm), UV-B (280-315 nm) and UV-A (315-400 nm), of which UV-C and UV-B are the most energetic radiation (Rozema *et al.*, 1997). However, all the UV-C radiation and most of the shortest wavelength region of UV-B are filtered out by the atmospheric ozone layer before it reaches the earth's surface, while solar UV-A passes almost unaltered through the atmosphere. The ambient levels of UV-B and UV-A are variable and affected by altitude, latitude, season and time of the day as well as cloud patterns (Madronich *et al.*, 1998; Herman *et al.*, 1999). Thus, the UV climate changes as one move from the equator to the poles and from sea level to high mountains.

Although high UV-B levels may trigger non-specific pathways in plants resulting in general stress responses, most plants raised under natural UV-B levels are well protected from UV-radiation and little damage occurs under such conditions (Jansen & Bornman, 2012; ROBSON *et al.*, 2014). Thus, in contrast to earlier focus on UV-B as a damaging agent, a novel vision has emerged, emphasizing the regulatory properties of low, ecologically relevant doses of UV-B radiation, and the important role that these play at the cell and plant level. In this respect, UV-B acts as an environmental signal stimulating the expression of genes involved in UV-B protection of plants and UV-B-specific photomorphogenesis signalling pathways (Jenkins, 2009; ROBSON *et al.*, 2014).

Light quality is sensed by different photoreceptors in plants. Phytochromes mediate the responses to red (R) and far-red (FR) light, whereas cryptochromes and phototropins are important sensors of UV-A and blue (B) light. Recently UV RESISTANT LOCUS 8 (UVR8) was shown to act as an

UV-B sensor (Rizzini *et al.*, 2011). Light-induced changes in plant growth and development are complex and known to be regulated through multiple pathways. UVR8 interaction with CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1) leads to up-regulation of *HY5/HYH*, which encode transcription factors known to be involved in photomorphogenesis (Heijde & Ulm, 2012).

Several reports have demonstrated that ambient levels of UV-B affects plant morphology e.g. by reducing leaf expansion, hypocotyl and shoot elongation as well as apical dominance (e.g. Jansen, 2002, Jenkins, 2009; Robson *et al.*, 2014). In young leaves of *Arabidopsis thaliana* and expanded leaves of rice (*Oryza sativa*) the levels of the auxin indole-3-acetic acid (IAA) decreased in response to UV-B exposure (Huang *et al.*, 1997; Hectors *et al.*, 2012). In *A. thaliana* UV-B has also been shown to influence genes involved in auxin biosynthesis, conjugation and transport as well as auxin-responsive genes (Hectors *et al.*, 2007). In rice the UV-B-induced decrease in IAA levels in leaves was associated with increased IAA oxidase activity (Huang *et al.*, 1997). Thus, similar to different light qualities in the photosynthetic active part of the spectrum such as R, B and FR light (Behringer & Davies, 1992; Steindler *et al.*, 1999; Islam *et al.*, 2014) UV-B affects extension growth at least in leaves through action on IAA physiology. However, information on effects of UV-B on IAA content in stems is limited.

Several pathways for biosynthesis of IAA have been demonstrated in a range of plant species (e.g. Tivendale *et al.*, 2014). When first described in *A. thaliana*, *YUCCA* (*YUC*) genes were suggested to be rate-limiting in auxin biosynthesis (Zhao *et al.*, 2001). The roles of *YUC* genes have been debated lately, among others since small changes only in IAA levels have been observed in *YUC* overexpressing plants although IAA metabolites increased (Ross *et al.*, 2011; Tivendale *et al.*,

2014). Nevertheless, *YUC*-overexpressing plants show high-auxin phenotypes, and *YUC* gene expression have been shown to be affected by environmental conditions such as light quality and temperature (Tao *et al.*, 2008; Stavang *et al.*, 2009; Tivendale *et al.*, 2014). In pea two *YUC* genes *PsYUC1, PsYUC2* have been identified and have been shown to be expressed in different tissues like apical shoot tissue, mature leaves and developing seeds (Tivendale *et al.*, 2010).

Gibberellins (GAs) are diterpenoid acids acting as plant growth regulators by affecting a range of developmental processes in higher plants such as elongation growth, germination, dormancy, flowering and sex expression. Although GA is well known to control shoot elongation and leaf expansion (Chandler & Robertson, 1999; Richards *et al.*, 2001; Yamaguchi, 2008), information about involvement of GA in UV-B-responses is limited. The GA biosynthesis inhibitor paclobutrazol was shown to enhance tolerance to elevated UV-B levels with respect to photosynthesis effectivity in soybean (*Glycine max*) (Kraus *et al.*, 1995). Paclobutrazol treatment also increases the thickness of the leaves and the epicuticular wax layers compared to in untreated control plants, changes which are commonly also observed in response to UV-B (Jansen, 2002). Recently it was demonstrated that in young seedlings (7 days old) of *A. thaliana* transcript levels of the GA inactivation gene *GA2-oxidase 1* (*GA2ox1*) increased in response to UV-B exposure (Hayes *et al.*, 2014). Also, although the type of gene within each gene family was not specified and each gene family consists of several genes, in rosettes of *A. thaliana*, GA biosynthesis genes of the *GA3-oxidase* (*GA3ox*) and *GA20-oxidase* (*GA20ox*) types were generally down-regulated under UV-B, whereas a *GA2ox* was down-regulated and up-regulated in UV-B-adapted plants and plants exposed to acute UV-B, respectively (Hectors *et al.*, 2007). However, the information on interaction of UV-B with GA metabolism with respect to stem elongation is scarce.

A key role of GAs in stem elongation is evident from observing GA deficient mutants, which are much shorter than the corresponding wild types. About 136 GAs are identified and characterized so far in higher plants, fungi and bacteria (http://www.plant-hormones.info/gibberellins.htm). Most of these are precursors or inactive forms, and only few GAs are bioactive, i.e. GA_1, GA_3, GA_4, GA_5, GA_6, and GA_7 (Hedden & Phillips, 2000; Bottini *et al.*, 2004; Yamaguchi, 2008). Biosynthesis of GA differs with tissue type and developmental stage. In vegetative tissues of different species commonly either the early 13-hydroxylation pathway or the non-13-hydroxylation pathway dominates, resulting in the bioactive GA_1 and GA_4, respectively. In pea the early 13-hydroxylation pathway is the main pathway in which GA_1 is synthesized by conversion of GA_{20} by a *GA3ox*, encoded by the *LE* gene (Campell & Bonner, 1986; Lester *et al.*, 1997; Weller *et al.*, 2009; Reinecke *et al.*, 2013) (Fig. 1). Increasing the expression of the GA biosynthesis genes *GA20ox* and *GA3ox* or the GA inactivation genes *GA2ox* can increase or decrease shoot growth, respectively (Yamaguchi, 2008; Kurepin & Pharis, 2014). A wide range of studies have demonstrated that the GA levels are regulated through transcriptional up- or down-regulation of GA metabolism genes (Kamiya & García-Martínez, 1999; Hedden & Phillips, 2000; Yamaguchi, 2008). Furthermore, GA metabolism is subjected to feedback and feed-forward responses to GAs. Specifically, feedback-regulation with respect to *GA20ox* and *GA3ox* and feed-forward regulation of *GA2-oxidase* gene expression have been demonstrated (Hedden & Phillips, 2000; Oh *et al.*, 2006; Zhao, XY *et al.*, 2007).

In addition to the aforementioned effect of UV-B on GA metabolism genes in *A. thaliana*, GA biosynthesis and metabolism are known to be affected by environmental conditions like light quality and temperature. Several studies have demonstrated significant reduction in the level of the active GA_1 after exposure of plants, including pea, to B and R light and increase in GA in response

to FR light (Gil & García-Martinez, 2000; Hedden & Phillips, 2000; Olsen & Junttila, 2002; Reid *et al.*, 2002; Zhao, X *et al.*, 2007; Islam *et al.*, 2014). In pea B light was shown to down-regulate *GA20ox* and *GA3ox* and up-regulate *GA2ox* (Reid *et al.*, 2002).

Abscisic acid (ABA) levels in leaves have been shown to increase in response to UV-B radiation, like in maize (*Zea mays*) and grape vine (*Vitis vinifera*), and was shown to enhance formation of UV-B protecting compounds(Tossi *et al.*, 2009; Berli *et al.*, 2010). Furthermore, ABA was shown to act in protection against UV-B through interaction with nitric-oxide-mediated signalling (Tossi *et al.*, 2009). Although the significance of this is unclear, ABA levels were also found to be affected by light qualities affecting shoot elongation such as R and FR light, with lower ABA levels correlating with reduced plant height (Weatherwax *et al.*, 1996; Kurepin *et al.*, 2007; Islam *et al.*, 2014).

In the greenhouse industry control of shoot elongation and plant morphology is essential since small and compact ornamental plants and transplants require less space during cultivation, are easier to handle and transport, and are generally preferred by the consumers. Compact plants are commonly obtained by using plant growth regulators (growth retardants). However, due to their potential negative effects on human health and the environment (De Castro *et al.*, 2004; Sørensen *et al.*, 2009) several studies have addressed use of light quality and temperature for manipulation of plant morphology in greenhouse-grown plants (Hickman, 1986; Erwin *et al.*, 1991; Myster & Moe, 1995; Stavang *et al.*, 2005; van Ieperen, 2012; Islam *et al.*, 2014). In temperate areas exposure to lower day than night temperature or a temperature drop for a few hours, obtained by opening greenhouse vents, are commonly used to produce compact ornamental plants and transplants without a delay in production time (Myster & Moe, 1995; Stavang *et al.*, 2007). Thus, thermoperiodic responses of

plants, defined as all effects of a temperature differential between light and dark periods on responses of plants (Went, 1944), are exploited in this respect. However, in warmer periods and areas, sufficient temperature reduction for efficient regulation of shoot elongation is not possible to obtain by opening greenhouse vents, and growth regulators are still extensively used. Thermoperiodic control of stem elongation is associated with alterations in hormone contents. In *A. thaliana* IAA was found to be reduced under lower day than night temperature compared to the opposite temperature regime (THINGNAES *et al.*, 2003). Furthermore, temperature alteration in light affects GA levels, particularly through action on GA inactivation genes, such as *GA2ox2* in pea and *GA2ox1* in *A. thaliana* (Grindal *et al.*, 1998; Stavang *et al.*, 2005; Stavang *et al.*, 2007; Yamaguchi, 2008; Stavang *et al.*, 2009). In pea a temperature drop in light or a lower day than night temperature, which reduce elongation growth and GA_1 contents, increase transcript levels of *PsGA2ox2* compared to a temperature drop during the night or higher day than night temperature (Grindal *et al.*, 1998; Stavang *et al.*, 2005; Stavang *et al.*, 2007; Stavang *et al.*, 2010). Thus, the thermoperiodic response is apparently meditated through affecting GA deactivation.

Although UV-B has been shown to affect the levels of auxin and ABA in leaves (Huang *et al.*, 1997; Tossi *et al.*, 2009; Berli *et al.*, 2010; Hectors *et al.*, 2012), information on effects of UV-B on hormone contents and metabolism, particularly with respect to GAs, in relation to stem elongation and leaf expansion is still limited. Using pea as a model plant we aimed at evaluating the effect of UV-B on metabolism of GA, IAA and ABA as related to stem elongation and leaf expansion. Also, since the combination of a daily temperature drop treatment and UV-B might be interesting as a tool for controlling plant morphology in greenhouses, in addition to effects of UV-B under constant temperature, effects of UV-B during a daily temperature drop was investigated.

Materials and methods

Plant materials and pre-growing conditions

Seeds of pea (*Pisum sativum* L.), cv Torsdag) were sown in 11 cm pots containing a standard fertilized sphagnum peat (Tjerbo Torvabrikk, Rakkestad, Norway) and perilite (3:1 w/w). The pre-treatment cultivation (except in the gibberellin (GA) application experiment) was done in a greenhouse compartment at The Centre of Plant Research in Controlled Climate (SKP), at Norwegian university of life sciences (NMBU), Ås, Norway (59°39'47''N 10°47'38''E). During the pre-cultivation period a temperature of 20℃ and relative humidity at 70% and both natural and supplemental light during a photoperiod of 16 h was used. The supplemental light from high pressure sodium (HPS) lamps (Osram NAV T-400W, Munich, Germany) was turned on when the natural light was below 200 µmol m^{-2} s^{-1}. The supplemental irradiance was 100 (±10) µmol m^{-2} s^{-1} measured with a quantum sensor (Model L1-185, Li-COR inc, Lincoln, NE, USA). In the GA-application experiments, the pre-treatment cultivation was done in growth chambers (manufactured by SKP). Light was then provided by fluorescent tubes with a photosynthetic photon flux density (PPFD) of 100 µmol m^{-2} s^{-1} (MASTER TL-D Super 80 36W/840 Philips, Eindhoven, The Netherlands), and a red: far-red (R:FR) ratio of 1.7 was achieved through addition of light from incandescent lamps (Osram). The plants were exposed to a 12 h photoperiod and a constant temperature of 20ºC. Air humidity was increased by trays with water was placed beneath the perforated chamber floors (Stavang et *al.*, 2005). In all cases, the pre-treatment cultivation ended after 6 days when the plant had 1-1.5 cm long shoots.

Experimental conditions

At day 6 after sowing uniformly sized seedlings were transferred to the aforementioned growth chambers with light conditions as described above, and subjected to UV-B and temperature drop treatments. During this experiment all plants were grown at the same daily mean temperature of 20℃ but under two different temperature regimes; either constant temperature (CT) at 20℃ or a daily temperature drop (TD) treatment from 21℃ to13℃ for 6 h in the middle of the 12 h light photoperiod and otherwise 21℃. For each of these two temperature regimes, a subset of plants was exposed to UV-B for 6 h and another subset served as control plants not exposed to UV-B. Three UV-B fluorescent tubes (UVB-313, Q-Panel Co., Cleveland, OH, USA) were used in each UV-B treatment. The UV-B treatment was applied for 6 h in the middle of the light period simultaneously with the TD. A 0.13 mm thick cellulose diacetate foil (Jürgen Rachow, Hamburg, Germany) was used to filter the shortest part of the UV-wavelengths, i.e. wavelengths below 290 nm. The cellulose acetate film was put 10 cm under the UV-B lamps. The irradiance from the UV-B tube was measured on the top of the plant once simultaneously with a broadband UV-B sensor (SKU340, Skye Instruments, Powys, UK) and an Optronic model 756 spectroradiometer (Optronic laboratories, Orlando, FL, USA). Based on the calibration factor from this comparison, the absolute UV-B irradiation in the chamber measured with the broadband sensor was 0.45 W m^{-2}. During the temperature drop the UV-B level was reduced with approximately 25% due to reduced efficiency of the lamps at low temperature.

Recording of morphological parameters

Plant growth and morphology were monitored by counting the number of leaves, measuring plant height and internode length as well as calculating total and specific leaf area (SLA). From each chamber 6 plants were used for the measurement of these parameters. The total shoot length was measured from the base of the plant to the shoot apex at day 0, 3, 6 and 10 after the start of the treatments. The distance between alternating leaves was measured to determine the length of internodes. All fully opened and mature leaves were counted. At the end of the experiment (day 10) fully expanded, mature leaves from 6 plants were collected and the leaf area determined using a leaf area meter (Model LI-3100, Li-Cor, Lincoln, NE, USA). Fresh weight of leaves was measured, and the dry weight determined after drying in an oven at 70°C for 5 days. SLA ($cm^2\ g^{-1}$) was calculated according to (Vile *et al.*, 2005) as the ratio between the leaf area and dry weight (DW) of the 2^{nd} leaf as counted from the basis of each plant. Three independent, repeated experiments with 6 plants in each were performed.

Exogenous application of GA_3

To evaluate if exogenously applied gibberellic acid (GA_3) could counteract the impact of UV-B either when provided separately or in combination with temperature drop treatment, in one experiment GA_3 (Sigma-Aldrich, St. Louis, MO, USA) was applied to the shoot apex, and in another independent experiment GA_3 was applied to the first unfolded leaf. For apex application 10 µg GA_3 in 1 µl 96% ethanol or 1 µl of 96% ethanol only (mock treatment) were used for each of ten plants. For leaf application 10 µg GA_3 per 10 µl 96% ethanol or 10 µl 96% ethanol only

(mock treatment) were applied to each of ten plants. GA_3 and ethanol application was done at the start of the UV-B treatment for apex application, and for leaf application on the second day of the UV-B exposure, when the first leaf had unfolded. Plant response was monitored during the growth period for eight days by measuring plant height from the base of the plant to the apex.

Plant hormone analysis

At day 10 of the daily UV-B treatment, the uppermost, elongating 5-10 cm of the shoot tip was harvested into liquid nitrogen in the middle of the light period. This corresponded to three hours into the daily UV-B treatment (and three hours into the temperature drop exposure in the case of temperature drop). At harvest, leaves and stem tissue including the apical meristem were separated and put into different tubes. For each tissue type three repeated samples each consisting of 6 plants were collected from each treatment, freeze dried and stored in -80℃ before freeze drying.

Chemicals and calibration curves

A number of compounds, namely dihydrophaseic acid (DPA), phaseic acid (PA), ABA glucose ester (ABA-GE), 7'- OH-ABA, neoPA, *trans*-abscisic acid (*trans*-ABA) and indole-3-acetic acid-glucose conjugate (IAA-Glu) were synthesized and prepared at the Plant Biotechnology Institute of the National Research Council of Canada (PBI-NRC Saskatoon, SK, Canada). ABA, IAA-Leu, IAA-Ala, IAA-Asp and IAA were purchased from Sigma-Aldrich. Gibberellic acid (GA) 1, 3, 4, 7, 8, 9, 19, 20, 24, 29, 44, and 53 were purchased from the Research School of Chemistry,

Australian National University (Canberra, Australia). Deuterated (d) forms of the hormones were used as internal standards. d_3-DPA, d_5-ABA-GE, d_3-PA, d_4-7'-OH-ABA, d_3-neoPA, d_4-ABA, d_4-*trans*-ABA, d_3-IAA-Leu, d_3-IAA-Ala, d_3-IAA-Asp, d_3-IAA-Glu and $^{13}C_4$-IBA were synthesized and prepared at PBI-NRC according to (Abrams *et al.*, 2003) and (Zaharia *et al.*, 2005). d5-IAA was purchased from Cambridge Isotope Laboratories (Andover, MA, USA) and d_2-GAs 1, 3, 4, 7, 8, 9, 19, 20, 24, 29, 34, 44, 51 and 53 were purchased from the Research School of Chemistry, Australian National University. The deuterated forms of selected hormones used as recovery (external) standards, d_6-ABA and d_2-ABA-GE, were prepared and synthesized at PBI-NRC. Calibration curves were created for all compounds of interest. Quality control samples (QCs) were run along with the tissue samples.

Hormone quantification by HPLC-ESI-MS/MS

The procedure for quantification of multiple hormones and metabolites, including auxin and metabolites (IAA, IAA-Asp and IAA-Glu), ABA and metabolites (ABA, PA, DPA, 7'-OH-ABA, neoPA and ABA-GE) and different GAs has been described in detail by (Chiwocha *et al.*, 2003; Chiwocha *et al.*, 2005). Also, levels of cytokinins and cytokinin metabolites were analysed as described by these authors and Islam *et al.* (2014), but since these compounds could be detected in a few cases only, results were inconclusive and thus not included here. For the hormone analyses, 50 mg of each sample was weighed, extracted and purified (also described in Islam *et al.*, 2014). The purified extracts were then injected onto a Genesis C18 HPLC column (100 x 2.1 mm, 4 μm, Chromatographic Specialties, Brockville, ON, Canada) and separated by a gradient elution of water against an increasing percentage of acetonitrile that contained 0.04% acetic acid. Briefly, the

analysis utilized the Multiple Reaction Monitoring (MRM) function of the MassLynx v4.1 (Waters Inc) control software. The resulting chromatographic traces were quantified off-line by the QuanLynx v4.1 software (Waters, Mississauga, ON, Canada) wherein each trace was integrated and the resulting ratio of signals (non-deuterated/deuterated internal standard) was compared with a previously constructed calibration curve to yield the amount of analyte present (ng per sample). Calibration curves were generated from the MRM signals obtained from standard solutions based on the ratio of the chromatographic peak area for each analyte to that of the corresponding internal standard, as described by (Ross *et al.*, 2004). The quality control (QC) samples, internal standard blanks and solvent blanks were also prepared and analysed along each batch of tissue samples.

Analysis of transcripts of hormone metabolism

At day 10 of daily UV-B treatment, three hours after start of the UV-B exposure (= the middle of the light period), three repeated samples, each consisting of the elongating part of the shoot tips (about 5-10 cm) from 6 plants from each treatment, were collected and their leaves and stem were put in separate tubes. The samples were immediately frozen in liquid nitrogen and stored at -80℃ until the RNA was extracted.

Total RNA were extracted from 100 mg of homogenized tissue per sample using RNeasy Plant Mini Kit (Qiagen, D-40724, Hilden, Germany). RNA purification were carried out with Pure Link™ RNA Mini Kit (Life technologies, Carlsbad, CA, USA). Any remaining DNA was removed with TURBO DNA-free™ Kit (Applied Biosystems, Foster City, CA, USA). The concentration of total RNA was analysed with a NanoDrop ND-1000 Spectrophotometer (Termo Scientific,

Wilmington, DE, USA) and integrity analysed with an Aglient 2100 bioanalyzer (Aglient Technologies, Palo Alto, CA, USA). 1000 ng of total RNA from each sample was reverse-transcribed using SuperScript III First-Strand Synthesis SuperMix for qPCR (Invitrogen, Carlsbad, CA, USA). However, for analysing *GA2ox2*, *YUC1* and *YUC2* total RNA samples was reverse-transcribed using VILO kit (Invitrogen).

Primers and gene-specific TAMRA probes for GA metabolism genes were as described by (Stavang *et al.,* 2005). Primers for the auxin biosynthesis genes *YUC1* and *YUC2* (Tivendale *et al.,* 2010) were designed using Primer 3 software (http://www.bioinformatics.nl/cgi-bin/primer3/primer3_www.cgi). Primers and Probes are listed in Table 2 and 3. Transcript levels were analyzed using 7500 Fast Real-Time PCR System (Applied Biosystems). All chemicals used in the qRT-PCR reactions followed the recommendation as specified in TaqMan Gene Expression Master Mix protocol (Applied Biosystems) for the GA metabolism genes, and SYBR Select master Mix protocol (Life, USA) for the auxin biosynthesis genes. However, for TaqMan gene expression, instead of using a 50 µl reaction volume in each well, we used a 25 µl reaction volume. The primer concentration used for TaqMan gene expression analysis (*α-tubulin, Ls, Lh, Na, GA2ox1, GA2ox2, GA20ox1*, *GA3ox1)* was 900 nM, and the probe concentration 200 nM (Table 2). The primer concentration used for SYBR gene expression (*YUC1, YUC2*) was 250 nM (Table 3). Relative transcript levels were determined using the method of (Pfaffl, 2001). *α-tubulin* was used as endogenous reference gene since its transcripts levels were stable under the experimental conditions. For each gene, all samples were related to the sample with constant temperature without UV-B treatment. qRT-PCR reactions were conducted in triplicate for each sample and a minus reverse transcriptase reaction was included to detect any remains of genomic DNA.

Statistical Analysis

To test for effects of the different treatments (control, UV-B, temperature drop, UV-B under temperature drop), a one-way analysis of variance (ANOVA) was performed for each tissue type using a completely randomized design, followed by Tukey's test (Minitab software versions 16.1.1, State College, Pennsylvania, USA). Differences with $p \leq 0.05$ were considered significantly different.

Results

Effect of UV-B radiation on plant morphology

A 6 h daily period of UV-B exposure (0.45 W m^{-2}) in the middle of the light period reduced shoot elongation significantly ($p \leq 0.05$) by on average 9%, compared to untreated control plants (Fig. 2). However, there was no significant effect on internode length (Table 3). Also, UV-B significantly reduced total leaf area of the plants by 35%. However, there was no significant effect on the number of leaves (Table 3). There was no significant UV-B induced reduction in specific leaf area (SLA). This level of UV-B did not result in any visible damage. However, exposure to UV-B at the same irradiance for 7 h or higher irradiances resulted in leaf curling and tissue damage (results not shown).

When UV-B was provided together with a daily temperature drop from 21 to 13°C, both for 6 h in the middle of the light period, shoot elongation was significantly reduced by on average 30% compared to the temperature drop only and 40% compared to control plant grown under constant temperature without UV-B treatment (Fig. 2, Table 3). Thus, although the UV-B levels under constant temperature and temperature drop could not be directly compared since the UV-B lamp efficiency decreased during the temperature drop period (25% decrease), UV-B apparently affects stem elongation more strongly under the temperature drop treatment than under constant temperature. The exposure to UV-B radiation under the temperature drop treatment resulted in significant reduction in internode length by 26% and 31%, respectively, compared to temperature drop only and constant temperature without UV-B. Leaf area was not significantly reduced by UV-B and temperature drop compared to temperature drop only (only a trend with on average 18% reduction) but compared to constant temperature without UV-B a 25% reduction in leaf area was observed. SLA was not significantly affected by UV-B provided together with the temperature drop exposure.

Effect of GA_3 on shoot elongation in UV-B exposed plants

To evaluate if exogenously applied gibberellic acid, GA_3 could counteract the impact of UV-B on shoot elongation, GA_3 was applied either to the shoot apex or the first unfolded leaf. In both cases, GA_3 application resulted in strongly stimulated elongation growth under UV-B compared to the mock control or un-applied plants (Fig. 3). Application of GA_3 counteracted the UV-B-induced inhibition of shoot elongation both when UV-B was provided under constant temperature and together with a daily temperature drop treatment.

Effect of UV-B on gibberellins

To assess whether the effect of UV-B on shoot elongation and plant morphology was associated with modulation of GA metabolism, GA levels were analysed in apical stem tissue and young leaves harvested at day 10 of the UV-B exposure. The early 13-hydroxylation pathway is known to be the dominating one in pea (Ingram *et al.*, 1984; Ross *et al.*, 1989; Grindal *et al.*, 1998; Stavang *et al.*, 2005) (Fig. 1) and in accordance with this, non-13-hydroxylated GAs were hardly detected with a few exceptions (GA_{24} and GA_{51}) only in a few samples. The analyses demonstrated a significant effect of the UV-B exposure on GA metabolism in both plant tissues ($p \leq 0.05$) (Figs. 4 and 5). UV-B significantly reduced levels of the bioactive GA_1 by about 59% and 69% in apical stem tissue and young leaves, respectively, compared to control plants not exposed to UV-B (Fig. 4). Also, the levels of the inactivation product GA_8 decreased by 29% (trend of decrease only) and 55% ($p \leq 0.05$) in young stem and leaf tissue, respectively (Fig. 4). The ratio of GA_8 to GA_1 increased significantly from 4.1 to 7.2 in stem tissue ($p \leq 0.05$) and showed a trend of increase from 5.8 to 8.7 in leaves (Fig. 5), suggesting higher rate of inactivation in plants exposed to UV-B than in the control plants. Also GA_{19} and GA_{20}, which are the precursors of the bioactive GA_1, was affected by UV-B. GA_{19} showed a significant 39% decrease in apical stem tissue, whereas GA_{20} decreased by 63% and 55%, respectively in young stem ($p \leq 0.05$) and leaf tissue (trend of decrease only) (Fig. 4). The ratio of GA_{20} to GA_{19} also decreased significantly from 21.3 to 4.3 in young leaves, indicating reduced GA biosynthesis (Fig. 5). GA_{44}, the precursor of GA_{19}, also decreased significantly by 38% in young stem tissue but not leaves (Fig. 4). The precursor of GA_{44}, GA_{53} could be detected in a few stem tissue samples but not at all in leaves, but values were too few to

evaluate possible differences. GA_{29}, which is formed by an inactivation side step from GA_{20}, increased significantly by 55% in leaves under UV-B exposure (Fig. 4), and the ratio of GA_{29} to GA_{20} increased significantly in leaves (from 1.2 to 4.2) as well as stem tissue (from 1.5 to 4.5) (Fig. 5), indicating increased activity of this inactivation side step under UV-B.

Compared to the constant temperature control, under temperature drop treatment (6 h) levels of GA_1 showed a trend of decrease (23%) in apical stem tissue (Fig. 4). When UV-B was provided for 6 h together with the temperature drop treatment, the levels of the bioactive GA_1 decreased by 54% and 64%, respectively, in apical stem tissue ($p \leq 0.05$) and young leaves (trend of decrease) compared to the temperature drop treatment, and 65% and 66% for stem and leaves, (both at $p \leq 0.05$) respectively, compared to the constant temperature (Fig. 4). Under the combined UV-B and temperature drop treatment, the levels of the inactivation product GA_8 were significantly reduced by 39% and 40%, respectively, in young stem and leaf tissue compared to temperature drop only and as compared to constant temperature by 61% and 60% in stem and leaves, respectively. Furthermore, compared to temperature drop only, the level of GA_{19} was significantly reduced by the combined UV-B-temperature drop exposure, i.e. by 37% in young stem tissue. In leaves there were no significant difference in GA_{19} levels between temperature drop-UV-B treatment and drop only or constant temperature. The levels of GA_{20} in young leaves showed a significant decrease by 60% in response to UV-B under temperature drop and a trend of decrease (61%) in apical stem tissue. The ratio of GA_{20} to GA_{19} in leaves decreased significantly from 19.2 under temperature drop to 6.0 when combined with UV-B, but there was no such difference in stem tissue (Fig. 5). Under temperature drop UV-B decreased the contents of GA_{44} significantly in apical stem tissue by 41% (Fig. 4). Furthermore, exposure to UV-B under the temperature drop resulted in increasing trends in the ratio of GA_{29} to GA_{20}, i.e. from 1.7 to 3.5 in the stem tissue (significant

at $p \leq 0.05$) and from 0.9-2.6 in the young leaves (trend of increase) (Fig. 5), indicating increased GA_{20} inactivation by the side step.

Effect of UV-B on IAA and IAA metabolites

Effects of the 6 h daily UV-B exposure on IAA and IAA-metabolites (Figs. 1 and 6) was also investigated. UV-B provided under constant temperature resulted in a significant decrease (35%) of IAA in young leaves compared to control plants grown without UV-B (Fig. 6). However, in apical stem tissue there was no significant difference in IAA levels. For plants grown under constant temperature there were no statistically significant effect of UV-B treatment on the individual IAA conjugates IAA-Asp and IAA-Glu, but in leaves there was a significant increase in ratio of total IAA-conjugates to IAA (4.9 to 8.5), indicating increased IAA-conjugation (IAA-Asp and IAA-Glu) under UV-B treatment.

Under the temperature drop treatment, there was no significant reduction in IAA levels in apical stem tissue or young leaves after daily UV-B exposure, only a slight trend of decrease (27%) in young leaves (Fig. 6). There was no significant effect of combined UV-B and temperature drop exposure on the IAA-conjugates IAA-Asp and IAA-Glu, but in leaves an increasing trend in ratio of the sum of these IAA-conjugates to IAA (5.9 to 7.9) was observed between the combined treatment and temperature drop only.

Effect of UV-B on ABA and ABA metabolites

Under constant temperature ABA levels showed a significant decrease in apical stem tissue (42%) and a trend of decrease only in young leaves (36%) in response to the daily UV-B treatment (Fig. 7). There was no significant effect ($p \leq 0.05$) of UV-B on the ABA inactivation product PA (Figs. 1 and 7). However, the levels of DPA, which is formed from PA, decreased significantly in the young leaves (56%) and showed a trend of decrease in apical stem tissue (37%) in response to UV-B exposure. Another inactivation product neo-PA decreased significantly in apical stem tissue (60%) of UV-B treated plants, whereas still another inactivation product, 7`-hydroxy-ABA, showed trends of decrease only in young stem tissue (61%) and leaves (57%). The ABA-conjugate ABA-GE could be detected in some of the samples only, thus no conclusive results as to the effect of UV-B on its content were obtained.

When UV-B was provided daily under a temperature drop treatment, a significant decrease in ABA was observed in apical stem tissue (20%) (Fig. 7). For the recorded inactivation products there were no statistically significant differences between the combined UV-B-temperature drop treatment and temperature drop only, only trends of decreased levels similar to the effects of UV-B under constant temperature.

Effect of UV-B on hormone metabolism genes

To further investigate the regulation of GA metabolism by UV-B when provided under constant temperature or during a daily temperature drop treatment, transcript levels of GA metabolism genes

in apical stem tissue and young leaves of pea plants were analysed by real-time quantitative PCR in samples harvested three hours into the temperature drop or combined treatment after 10 days of daily exposure. Under constant temperature no consistent effect of UV-B was observed for transcript levels of *Ls*, *Lh* and *Na*, which encode enzymes acting early in GA biosynthesis (Figs. 1 and 8). Also, under this temperature regime no consistent effect of UV-B on the transcript levels of GA biosynthesis genes acting later in the pathway, *GA20ox1* and *GA3ox1* could be detected (Figs. 1 and 8). Furthermore, under constant temperature there was generally no statistically significant effect ($p \leq 0.05$) of UV-B on transcripts levels of the GA inactivation genes *GA2ox1* and *GA2ox2*, only trends of increase in the young leaves.

Also for UV-B provided under the daily temperature drop there were generally no statistically significant effects on transcript levels of the genes early in the GA biosynthesis pathway, except for a significant increase ($p \leq 0.05$) for *Na* in apical stem tissue (Fig. 8). Furthermore, there was a slight, but significant increase in transcript levels of *GA20ox1* in the young stem tissue under the combined UV-B-temperature drop treatment compared to temperature drop only, whereas no significant differences were found for *GA3ox1*. For the two GA inactivation genes *GA2ox1* and *GA2ox2* increasing trends only were observed in leaves when UV-B was given under the daily temperature drop treatment.

To investigate whether there was an effect of UV-B on *YUC* genes, which have been suggested to play a role in biosynthesis of IAA, we analysed the transcript levels of *YUC1* and *YUC2* genes in apical stem tissue and young leaves of pea plants exposed to UV-B under constant temperature and daily temperature drop treatment. There were no significant differences in transcript levels of any

of the two *YUC* genes in any of the two tissues, except a possible slight trend of reduced *YUC1* transcript level under UV-B (Fig. 9).

Discussion

In this study we have demonstrated that UV-B-induced inhibition of stem elongation and leaf expansion in pea are associated with alterations in the GA metabolism, resulting in decreased levels of active GA (GA_1) in apical stem tissue and young leaves. The decreased GA_1 levels are apparently a consequence of increased GA inactivation and probably also reduced GA biosynthesis. UV-B exposure was also found to result in decreased level of IAA in young leaves but not in apical stem tissue.

Effect of UV-B radiation on plant morphology

Significant inhibition of shoot elongation (9%) and leaf expansion (35%) in pea exposed to 6 h daily UV-B levels not resulting in visual damage (Fig. 2; Table 3), is consistent with UV-B responses reported for a wide range of plant species (Jansen, 2002; ROBSON *et al.*, 2014). Although not recorded in the present study, these morphological responses must obviously be associated with differences in cell number or cell size or a combination of both. It is well known that unlike extension of hypocotyls and cotyledons, elongation of proper stems also involves cell division in the subapical (rib) meristem, and leaf expansion involves substantial cell division activity (Sachs, 1965; Donnelly *et al.*, 1999). Indeed, UV-B-induced reduction of plant height and leaf area has previously been attributed to reduction in cell length (Ballaré *et al.*, 1991; Ballaré *et al.*, 1995; Liu *et al.*, 1995; Gonzalez *et al.*, 1998; Kim *et al.*, 1998; Kakani *et al.*, 2003; Hectors *et*

al., 2010) . UV-B has also been shown to inhibit cell division through action on cell cycle progress (Dickson & Caldwell, 1978; Nogués *et al.*, 1998; Wargent *et al.*, 2009; Jiang *et al.*, 2011; Biever *et al.*, 2014). Unlike the situation in a range of studies, but like that of others (ÅLENIUS *et al.*, 1995; Johanson *et al.*, 1995; Jansen, 2002) specific leaf area (leaf thickness) was not affected by UV-B in our study (Table 3). Nevertheless, taken together, like demonstrated in a range of species, UV-B exposure reduces the surface area of pea plants.

When UV-B was provided together with a daily 6 h temperature drop treatment from 21 to 13ºC (mean daily temperature of 20ºC like for constant temperature treatment), stem elongation was inhibited substantially, i.e. 30% compared to temperature drop only and 40% compared to constant temperature (Fig. 2). Thus, although the UV-B levels were not identical in the two temperature treatments due to about 25 % reduced efficiency of the UV-B tubes after two hours of temperature drop treatment, the larger UV-B-induced inhibition of stem elongation in the combined treatment, indicates a synergistic effect of UV-B and lowered temperature. A daily temperature drop treatment or lower day than night temperature is well known to reduce shoot elongation in a range of species including pea, and are commonly used as tools for controlling shoot elongation in greenhouses in northern areas (Moe *et al.*, 1992; Myster & Moe, 1995). Although the combined effect of temperature drop and UV-B apparently may be interesting as a tool to efficiently control shoot elongation in greenhouses without using chemical plant growth regulators, great care must be taken to avoid UV-related damage. It is well known that the activity of the enzyme photolyase, which is involved in repair of UV-induced DNA damage, is affected by temperature with decreasing activity with decrease in temperature (Pang & Hays, 1991).

UV-B reduces the bioactive GA_1 but not the IAA content in apical stem tissue

The fact that application of GA_3 counteracted the inhibitory effect of UV-B on stem elongation in pea strongly indicates a UV-B-induced alteration of GA metabolism (Fig. 3). This was confirmed by measurement of the endogenous GA levels (Fig. 4). Under both temperature regimes, the levels of the bioactive GA_1 decreased significantly in apical stem tissue in response to 6 h daily UV-B exposure. Consistent with the differences in shoot elongation, there was a slight trend of lower GA_1 level under the combined treatment with UV-B and temperature drop (65% reduction) compared to UV-B treatment under constant temperature (59% reduction). GA is well known to affect stem elongation by acting on cell division and cell elongation in the subapical meristem (Sachs, 1965; Jones & Kaufman, 1983; Sauter *et al.*, 1995; Hansen *et al.*, 1999). Since quite large parts of shoot tips were harvested (5-10 cm), i.e. all internodes still elongating to a larger or smaller degree, larger differences in GA_1 levels might have been observed if the analyses were more targeted to the region of maximal cell division and cell elongation. A gradient of GA_1 in shoot apices has been observed with highest GA_1 levels in the region of highest cell division frequency just beneath the shoot tip (Olsen *et al.*, 1995; Hansen *et al.*, 1999). In pea (Yang *et al.*, 1993) reported the largest effect of GA in internodes less than 25% expanded. Although cell elongation and cell division in the subapical meristem were not recorded in the present study, action of UV-B on cell elongation and cell division in shoot tips of pea plants like in other species (Liu *et al.*, 1995; Kim *et al.*, 1998; Kakani *et al.*, 2003; Wargent *et al.*, 2009; Hectors *et al.*, 2010; Jiang *et al.*, 2011; Biever *et al.*, 2014), is apparently mediated through reduction of the content of the bioactive GA_1. Earlier studies have demonstrated reduction in levels of bioactive GA in apical stem tissue in response to

temperature drop treatment or lower day than night temperature, including in pea (Grindal *et al.*, 1998; Stavang *et al.*, 2005; Stavang *et al.*, 2007). Here such a trend was also observed, and as stated above, UV-B decreased the GA_1 levels further when provided under the 6 h daily temperature drop period.

Significantly reduced levels of the precursors of the bioactive GA, i.e. GA_{44} GA_{19}, GA_{20}, and the first inactivation product GA_8, in apical stem tissue in response to daily UV-B treatment, independently of temperature (Figs. 1 and 4), might indicate that GA biosynthesis as well as GA inactivation are affected by UV-B. Inspection of the ratio of GA_{44}, GA_{19} or GA_{20} to their immediate precursors (Fig. 5) and the transcript levels of the GA biosynthesis genes (Figs. 1 and 8) did not reveal any consistent effect of UV-B on any GA biosynthetic step that could easily explain the decrease of GA_{44}, GA_{19} and GA_{20} in apical stem tissue. On the other hand, the ratios of the GA inactivation products GA_8 and GA_{29} to their precursors, GA_1 and GA_{20}, respectively, increased significantly in response to UV-B (Fig. 5). This supports that GA inactivation is enhanced by UV-B. Increased transcript levels of any of the GA inactivation genes *GA2ox1* and *GA2ox2* in the apical stem tissue should then also be expected, but this could not be detected (Fig. 8). However, exposure to temperature drop only significantly increased the transcript level of *GA2ox2* by about 3-fold in apical stem tissue (3 h into the temperature drop treatment) compared to constant temperature at the same daily mean temperature. Such an increase is consistent with earlier studies of pea exposed to temperature drop or lower day than night temperature (Stavang *et al.*, 2005; Stavang *et al.*, 2007). Also, transfer of *A. thaliana* seedlings from 20 to 29ºC under constant light resulted in decreased transcript levels of a *GA2ox*; i.e. *AtGA2ox1* (Stavang et al., 2009). It should be noted that several GA metabolism genes exhibit diurnal variation in their expression, and that of the two pea GA-inactivation genes studied, particularly *PsGA2ox*, shows a prominent diurnal variation (Stavang *et*

al., 2005). After twelve days under lower day than night temperature, *GA2ox2* transcript levels were very high during the first half of a 12 h photoperiod compared to under higher day than night temperature or constant temperature, all at the same daily mean temperature (Stavang *et al.*, 2005). However, later in the day, there were no differences in levels of this transcript in the three temperature regimes. In light of such diurnal variation, it cannot be excluded that transcript levels of the GA inactivation genes could be more clearly affected by UV-B at other time points than three hours into the 6 h daily UV-B exposure. Furthermore, although GA metabolism occurs in shoot apices, parts of the GAs present in apical stem tissue might also result from transport from leaves or other plant parts (King *et al.*, 2008; Yamaguchi, 2008). Accordingly, the levels of GAs and transcripts of GA metabolism genes in a specific plant part do not necessarily strictly correlate. Also, it cannot be excluded that different developmental stages of the harvested internodes might have masked any differences during specific developmental stages.

In addition to the effect of lowered temperature during the light phase on *GA2ox* genes, a *GA2ox* (*GA2ox7*) gene was previously shown to be induced by salt stress in *A. thaliana* (Magome et al., 2008). Collectively, these results and the increased ratios of the GA inactivation products GA_8 and GA_{29} to their precursors GA_1 (bioactive) and GA_{20} (precursor of GA_1), respectively, in apical stem tissue in the present study might imply a general role of GA inactivation in adjusting growth under conditions unfavourable for extensive shoot elongation. However, in line with the current view on UV-B, such adjustment of GA levels and accordingly reduced shoot elongation in response to ambient UV-B levels can be considered part of the adaptive behaviour of plants to the environment, rather than stressful (Hectors *et al.*, 2007).

Unlike demonstrated for IAA in leaves at least in *A. thaliana* and rice (Huang *et al.*, 1997; Hectors *et al.*, 2012), no effect of UV-B on IAA levels or the recorded IAA conjugates (IAA-Asp and IAA-Glu) in apical stem tissue was observed in the pea plants from the two temperature regimes (Fig. 6). Thus, although stem elongation like leaf expansion involve both cell division and cell extension, and IAA is known to enhance stem elongation in pea by stimulating cell extension (Sachs, 1965; Yang *et al.*, 1993; Yang *et al.*, 1996; Donnelly *et al.*, 1999), the obvious physiological differences between the two organ types may involve differences in hormonal regulation in response to specific environmental factors. Furthermore, the transcript levels of *YUC1* and *YUC2* were not significantly affected by UV-B or temperature drop exposure, except a possible slight trend of reduced *YUC1* transcript level under UV-B (Fig. 9). Is should be noted that although *YUC* genes have been suggested to be involved in IAA biosynthesis and are affected by light quality and temperature, their role in specific steps of IAA biosynthesis is currently debated (Tao *et al.*, 2008; Stavang *et al.*, 2009; Ross *et al.*, 2011; Tivendale *et al.*, 2014).

UV-B reduces contents of bioactive GA_1 and IAA in young leaves

The level of bioactive GA_1 in young leaves was reduced by a 6 h daily UV-B exposure in the middle of the light period when provided under constant temperature (59%, $p \leq 0.05$) and temperature drop treatment (23%, trend only) (Fig. 4). GA is well known to act in stimulation of leaf and petiole expansion (Jones & Kaufman, 1983; Chandler & Robertson, 1999; Richards *et al.*, 2001). The initial phases of leaf expansion involve cell division in addition to cell expansion, whereas in the late phase only cell expansion occur (Tsuge *et al.*, 1996; Donnelly *et al.*, 1999). The earlier demonstrated effects of UV-B on these basic growth processes (references above, reviewed in

Robson *et al.*, 2014) is thus likely to be at least partly mediated by the decreased content of GA_1. Significantly reduced contents of the first inactivation product GA_8 under both temperature regimes (55% under constant temperature, 40% under temperature drop) in response to UV-B, and significantly increased GA_{29} level (55%) under constant temperature, suggest that GA inactivation is affected by UV-B in young leaves like in apical shoot tissue. This is supported by the increased ratio of GA_{29} to GA_{20} (significant at $p \leq 0.05$ under constant temperature, trend of increase under temperature drop) and a trend of increase in ratio of GA_8 to GA_1 in young leaves of UV-B-exposed plants (Fig. 5). Although not statistically significant at $p \leq 0.05$, the trends of increased transcript levels of the GA inactivation genes *GA2ox2* and *GA2ox1* support increased GA inactivation in response to UV-B (Fig. 8). Whereas the GA2ox1 enzyme metabolise GA_{20} to GA_{29} and further to GA_{29} catabolite as well as GA_1 to GA_8, *GA2ox2* has a strong preference for GA_1 as substrate rather than GA_{20} (Reid *et al.*, 1992; Lester *et al.*, 1999). Thus, trends of increase in transcript levels of *GA2ox1* as well as *GA2ox2* in response to daily UV-B exposure is consistent with the increased ratios of GA_{29} to GA_{20} and GA_8 to GA_1. This is also consistent with previously demonstrated increase in transcript levels of *GA2ox1* in leaves of *A. thaliana* exposed to UV-B (Hayes *et al.*, 2014). Furthermore, the trend of decreased levels of GA_{20} (Fig. 4) and the significant reduction in the ratio of GA_{20} to its precursor GA_{19} in both temperature regimes (Fig. 5), indicates that GA biosynthesis in young leaves also is also affected by UV-B. However, there were no consistent trends in the transcript levels of the GA biosynthesis genes, which could explain reduced GA biosynthesis (Fig. 8). Like discussed above, transport of GAs from other plant parts might also have contributed to the GA content of the young leaves, and lack of correlation between GAs and transcript levels of GA metabolism genes. Also, since transcript levels of several GA metabolism genes commonly fluctuate on a diurnal basis, it cannot be excluded that analyses of other time points would have revealed such trends.

In contrast to in apical stem tissue, the level of IAA decreased in the young leaves of pea in response to the daily UV-B exposure (significant at $p \leq 0.05$ under constant temperature, trend only under temperature drop; Fig. 6). This is consistent with results from previous studies of *A. thaliana* and rice (Huang *et al.*, 1997; Hectors *et al.*, 2012). Although the reduction in IAA content was relatively limited in our study, even small changes in IAA levels can alter leaf extension, and regions with high cell division activity typically have high IAA levels, and areas of cell expansion lower IAA content (Ljung *et al.*, 2001). The reduced IAA level is consistent with the reduced cell division and cell expansion observed in leaves of UV-B exposed plants (Robson *et al.*, 2014 and references therein). The significant effect of UV-B on the ratio of the IAA conjugates recorded (IAA-Asp and IAA-Glu) to IAA in the young leaves in the present study (Fig. 6) indicates that the reduced IAA levels are due to enhanced conjugation. Indeed, an effect of UV-B on up-regulation of genes encoding IAA conjugation enzymes has been demonstrated in *A. thaliana* (Hectors *et al.*, 2007).

Levels of ABA and some ABA inactivation products are affected by UV-B

The content of ABA and the inactivation products of DPA and neo-PA in apical stem tissue were significantly lower in the UV-B-treated compared to the control plants (Fig. 7). Although reduced levels of ABA and ABA inactivation products in shoot tips were also reported earlier in plants exposed to light quality (R light) reducing plant height (Weatherwax *et al.*, 1996; Kurepin *et al.*, 2007; Islam *et al.*, 2014) the significance of such a reduction is unclear. In young leaves there were also trends of decrease in ABA in response to UV-B, and the inactivation product DPA was significantly reduced (Fig. 7). Such a trend of an UV-B induced decrease in ABA differs from earlier published results from leaves of species like maize and grape wine, where increase in ABA

in response to UV-B was shown to stimulate production of UV-B protecting compounds (Berli *et al.*, 2010; Tossi *et al.*, 2014). The reason for this difference remains elusive, but nevertheless, levels of certain flavonoids known to protect against UV-B, increased in response to UV-B also in the pea plants of the current study (unpublished results).

Conclusions

In this study UV-B-exposure was shown to reduce shoot elongation in pea plants more when provided under a daily temperature drop treatment than under constant temperature. UV-B-induced inhibition of shoot elongation and leaf expansion in pea was generally shown to be associated with modulation of GA metabolism in shoot apices and altered metabolism of GA and IAA in young leaves. Reduced level of the bioactive GA_1 in response to UV-B is apparently due to increased GA inactivation in both tissues, and probably also decreased biosynthesis, at least in leaves. Reduced level of IAA in leaves appears to be associated with increased IAA-conjugation.

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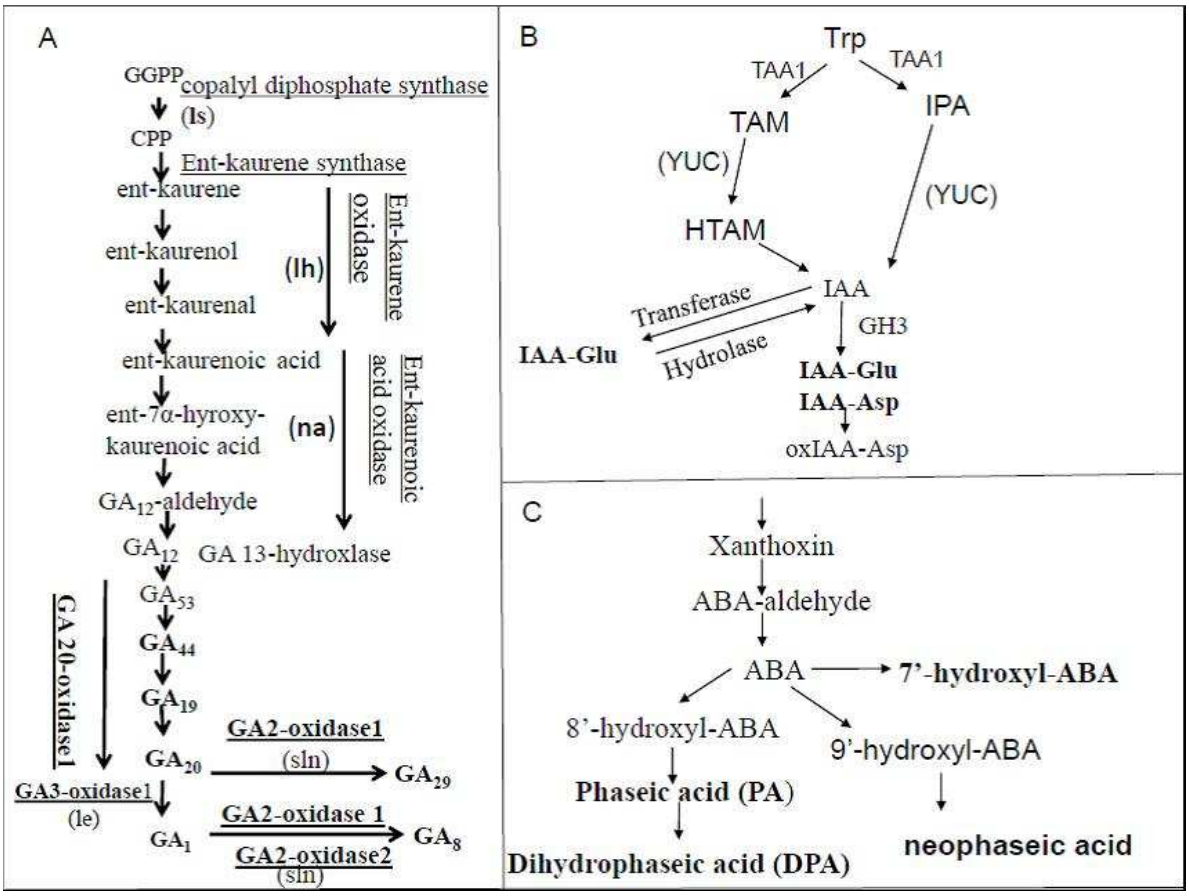

Fig. 1. Simplified hormone metabolism. (A) The early13-hydroxylation pathway of GA biosynthesis in vegetative tissue of pea. The GA metabolism genes in pea are underlined and corresponding GA mutants are given in parenthesis. (B) Possible tryptophan (Trp) dependent pathways (simplified) for indole-3-acetic acid (IAA) biosynthesis. Steps thought to be catalyzed by *YUCCA* (*YUC*) genes are shown. (C) Simplified biosynthesis pathway for abscisic acid (ABA). Bold letters indicate compounds detected in the young leaves and apical stem tissue of pea in this study. GGPP: Geranyl geranyl pyrophosphate; CPP: Copalyl pyrophosphate; IAA-glu: IAA-glutamate, IAA-Asp: IAA Aspartate, DPA: Dihydrophaseic acid, PA: Phaseic acid; Trp, Tryptophan; TAM, tryptamine; HTAM, N-Hydroxytryptamine; IPA, indole-3-acctamide. TAA1, Tryptophan aminostransferase of *Arabidopsis*1.

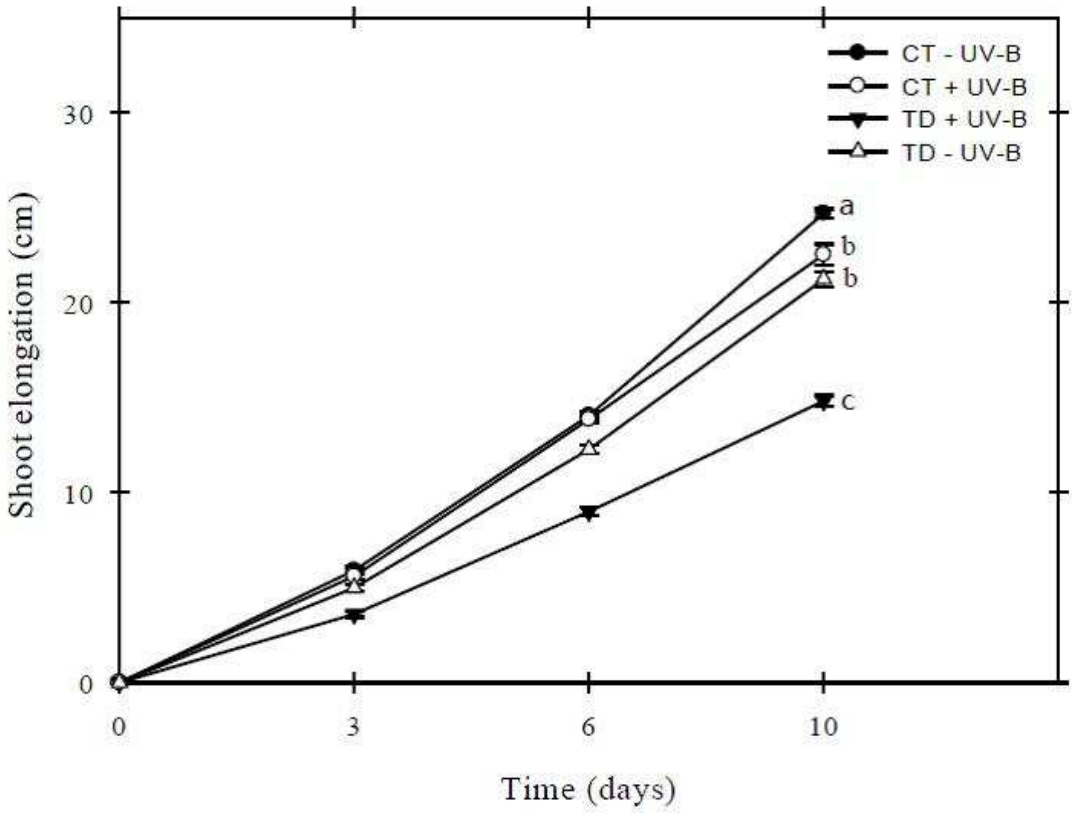

Fig. 2. Impact of 6 h daily UV-B exposure (+UV-B ; -UV-B: control plants not exposed to UV-B) in the middle of a 12 h photoperiod at two different temperature regimes; constant temperature (CT; 20℃) and a 6 h daily temperature drop (TD; 21 →13℃; same daily mean temperature as CT) on shoot elongation of pea plants. The results are mean ± SE of 6 individual plants in each of three repeated experiments (n=18). Different letters indicate significance differences at $p \leq 0.05$ (One-way ANOVA followed by Tukey`s test).

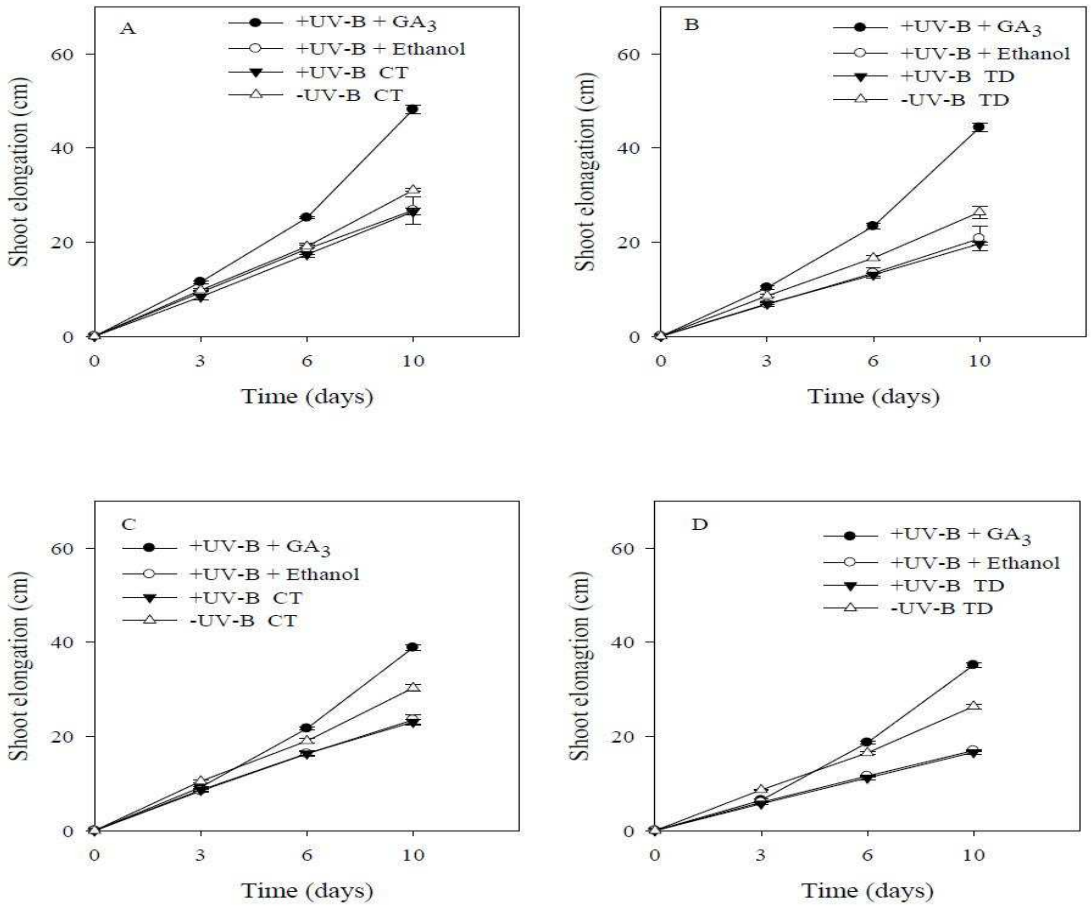

Fig. 3. Effect of application of gibberellic acid (GA_3) on shoot elongation of pea plants exposed to 6 h daily UV-B exposure (+UV-B= with UV-B; -UV-B = no UV-B) in the middle of a 12 h photoperiod under a constant temperature (20ºC; A, C) or under a 6 h daily temperature drop (TD; 21 →13℃; daily mean temperature 20ºC; B, D). 10 µg GA_3 (filled circle) was applied in two separate experiments per plants, either in 1µl 96% ethanol to apex (A, B) or 10 µl 96% ethanol to the first unfolded leaf (C, D). Application of ethanol only under UV-B = mock control. The results are the mean ± SE of 10 plants in each case.

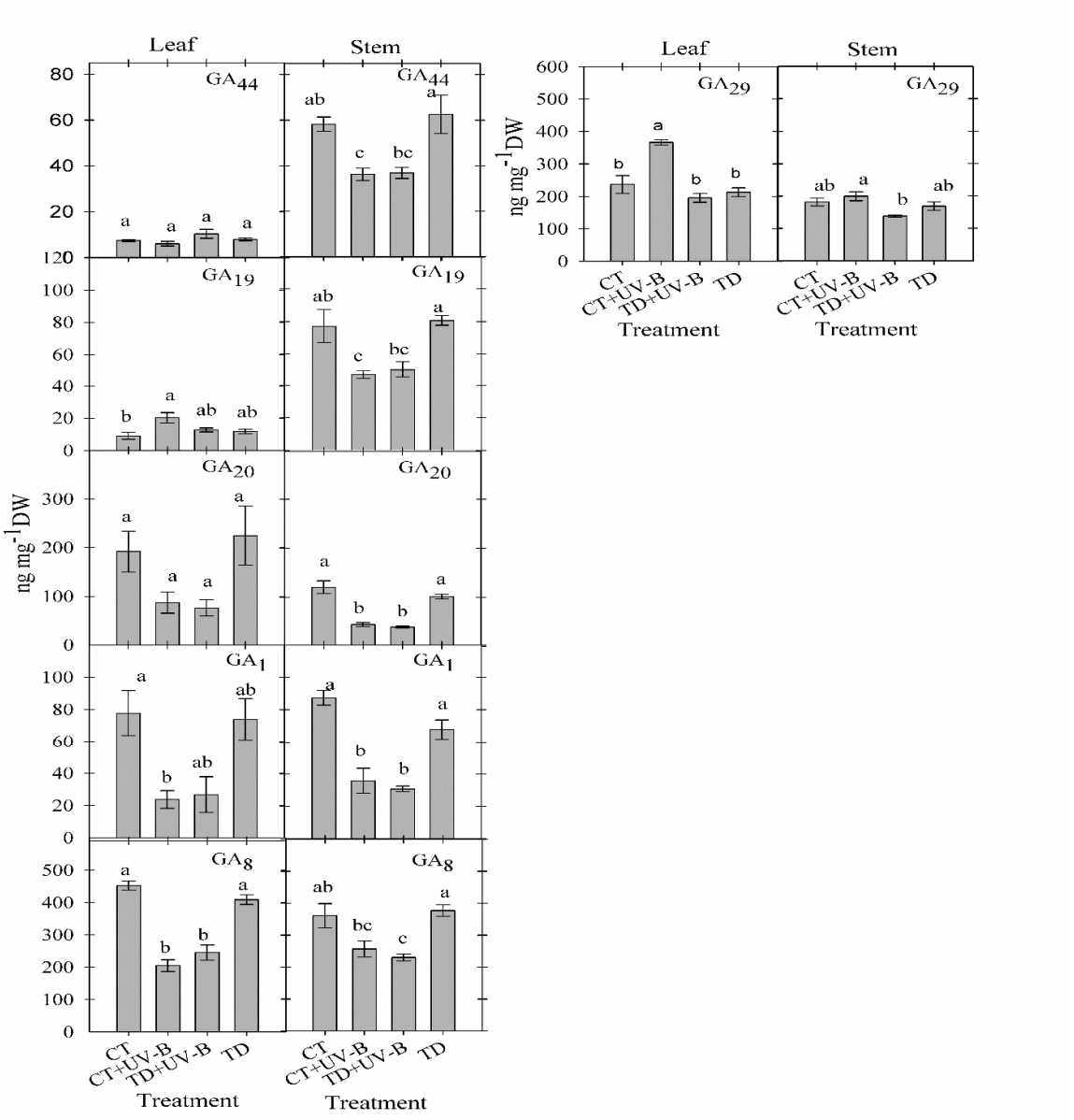

Fig. 4. Effect of 6 h daily UV-B treatment (+UV-B) in the middle of the light period for 10 days on gibberellin levels (GA) in pea plants under two temperature regimes (constant temperature (CT = 20ºC); and during a 6 h daily temperature drop (TD = 21→13ºC)). Results are the mean ± SE of three repeated samples, each consisting of 6 plants. Different letters indicate significant difference at $p \leq 0.05$ (One-way ANOVA followed by Tukey`s test).

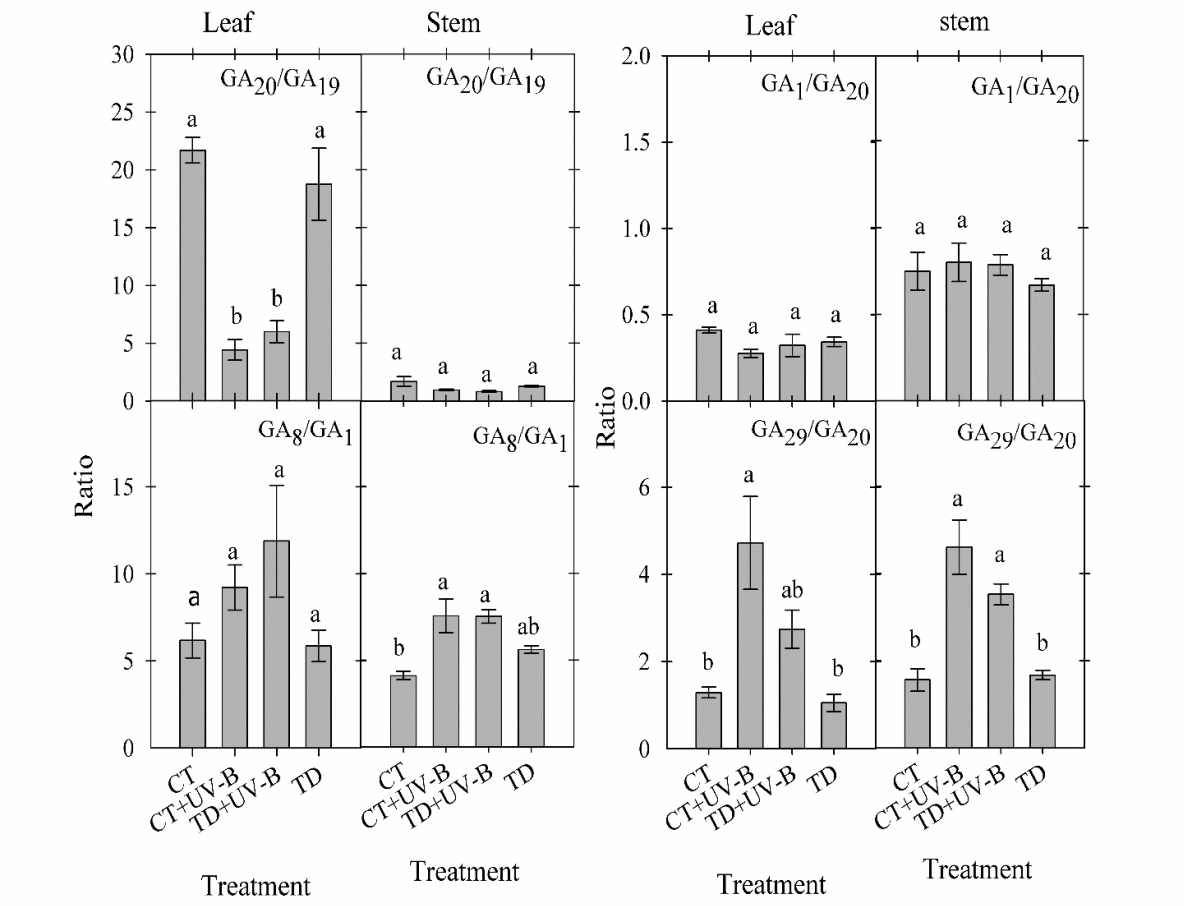

Fig. 5. Effect of 6 h daily UV-B treatment (+UV-B) in the middle of the light period for 10 days on the ratio of a specific gibberellin (GA) to its GA precursor in pea plants under two temperature regimes (constant temperature (CT = 20ºC); and during a 6 h daily temperature drop (TD = 21→13ºC)). Results are mean ± SE of three repeated samples, each consisting of 6 plants. Different letters indicate significant difference at $p \leq 0.05$ (One-way ANOVA followed by Tukey`s test).

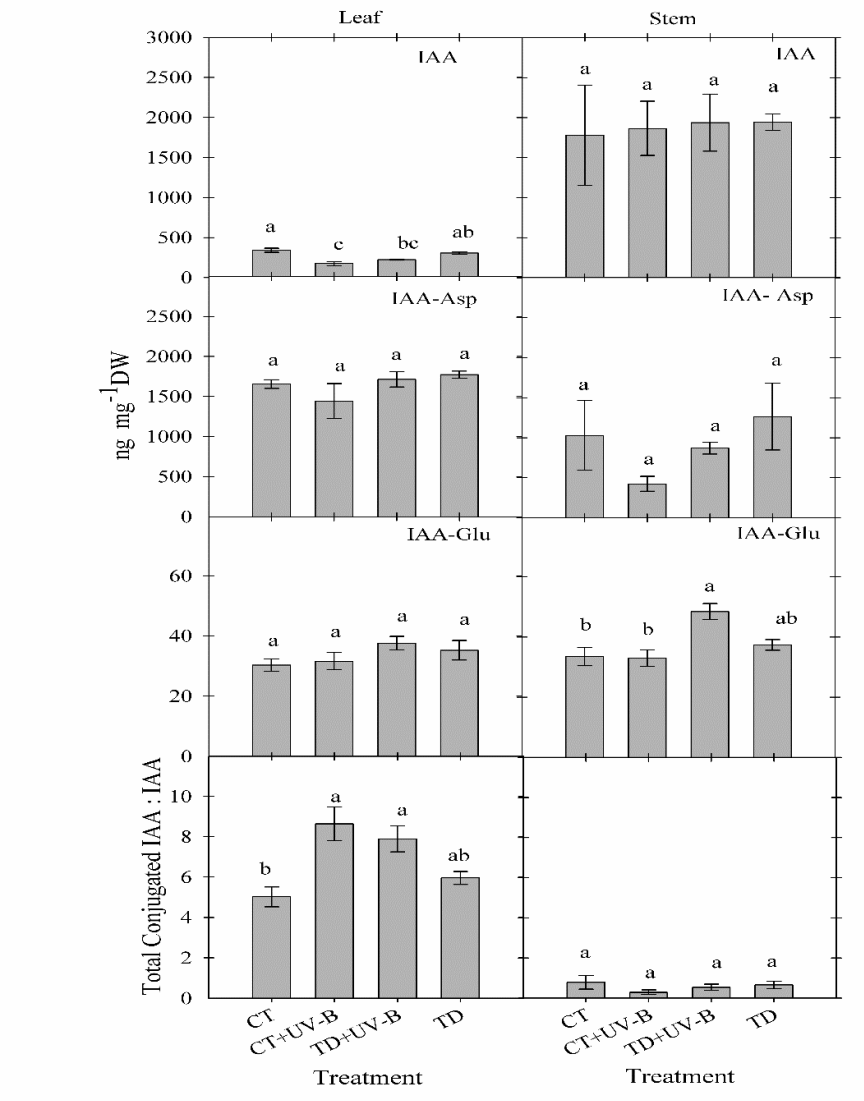

Fig. 6. Effect of 6 h daily UV-B treatment (+UV-B) in the middle of the light period for 10 days on levels of indole-3-acetic acid (IAA) and the IAA-conjugates IAA-Aspartate (IAA-Asp) and IAA-Glutamate (IAA-Glu) in pea plants under two temperature regimes (constant temperature (CT = 20ºC); and during a 6 h daily temperature drop (TD = 21→13ºC). Results are mean ± SE of three repeated samples, each consisting of 6 plants. Different letters indicate significant difference at $p \leq 0.05$ (One-way ANOVA followed by Tukey`s test).

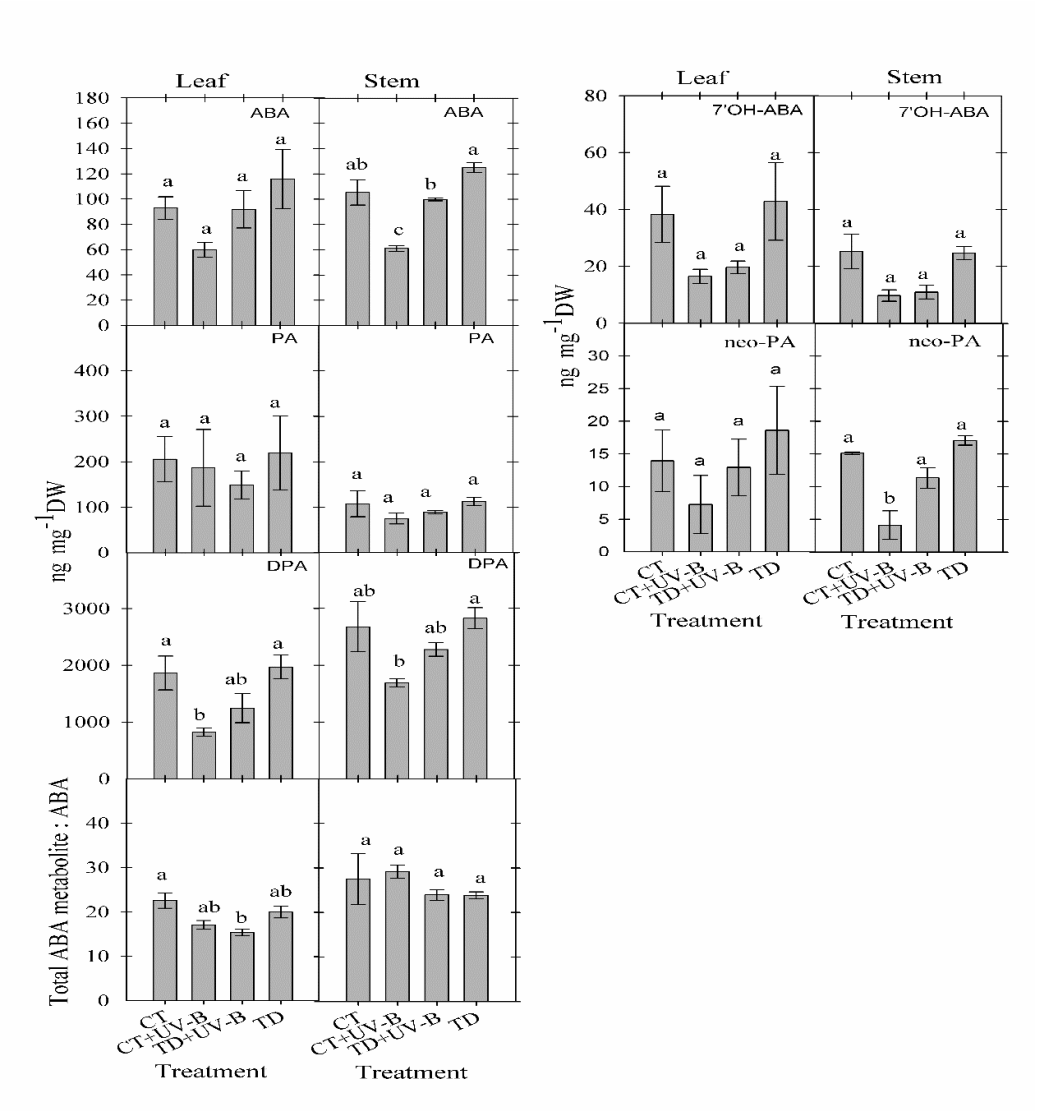

Fig. 7. Effect of 6 h daily UV-B treatment (+UV-B) in the middle of the light period for 10 days on levels of abscisic acid (ABA) and ABA metabolites in pea plants under two temperature regimes (constant temperature (CT = 20ºC); and during a 6 h daily temperature drop (TD = 21→13ºC)). PA: Phaseic acid; DPA: Dihydrophaseic acid. Results are mean ± SE of three repeated samples, each consisting of 6 plants. Different letters indicate significant difference at $p \leq 0.05$ (One-way ANOVA followed by Tukey`s test).

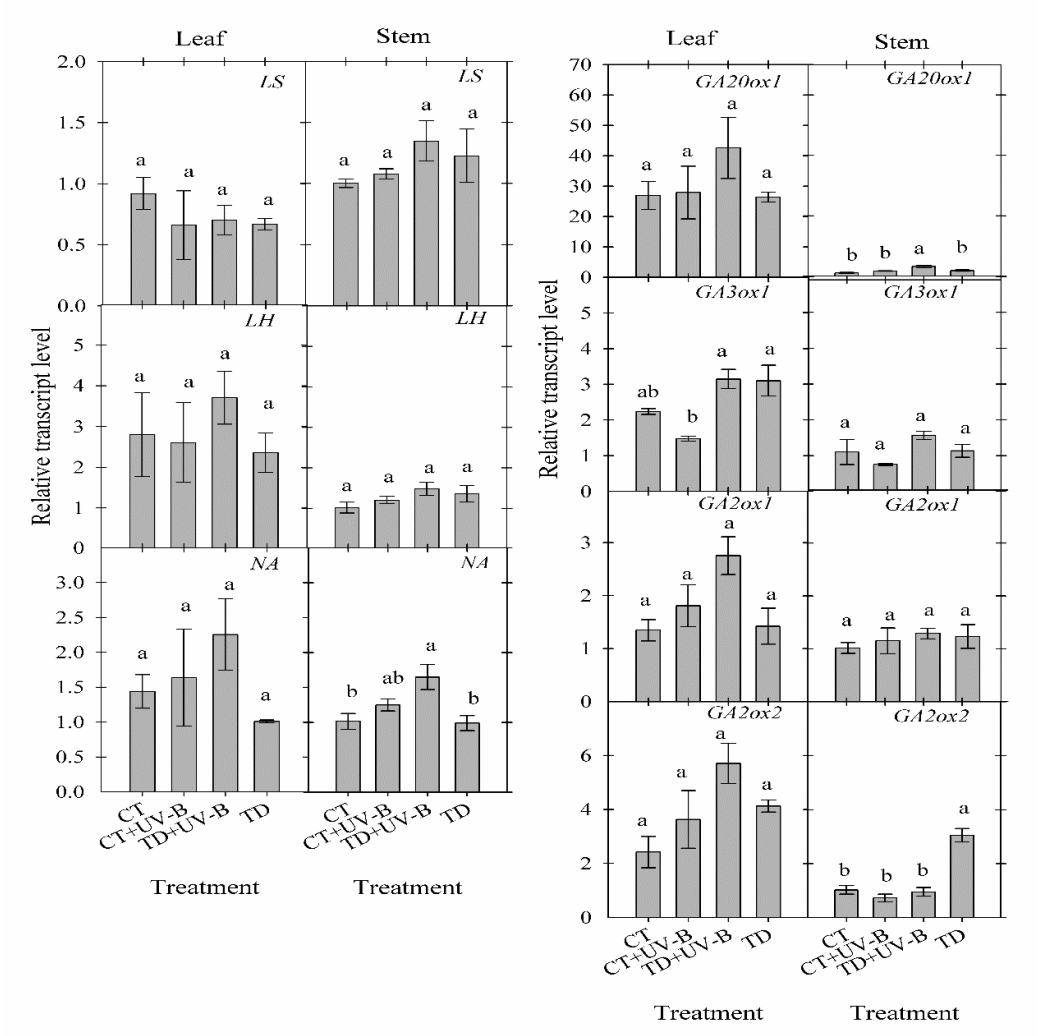

Fig. 8. Effect of 6 h daily UV-B treatment (+UV-B) in the middle of the light period for 10 days on transcript levels of early (*LS*, *LH*, *NA*) and late stage gibberellin (GA) metabolism genes (*GA20ox1*, *GA3ox1*, *GA2ox1* and *GA2ox2*) in pea plants under two temperature regimes (constant temperature (CT = 20ºC) and during a 6 h daily temperature drop (TD = 21→13ºC)). The transcript levels, normalized against α-tubulin, are shown as mean ± SE of fold change relative to CT in apical stem tissue. Three repeated samples, each consisting of 6 plants, were analyzed in each case. Different letters indicate significant difference at $p \leq 0.05$ (One-way ANOVA followed by Tukey`s test).

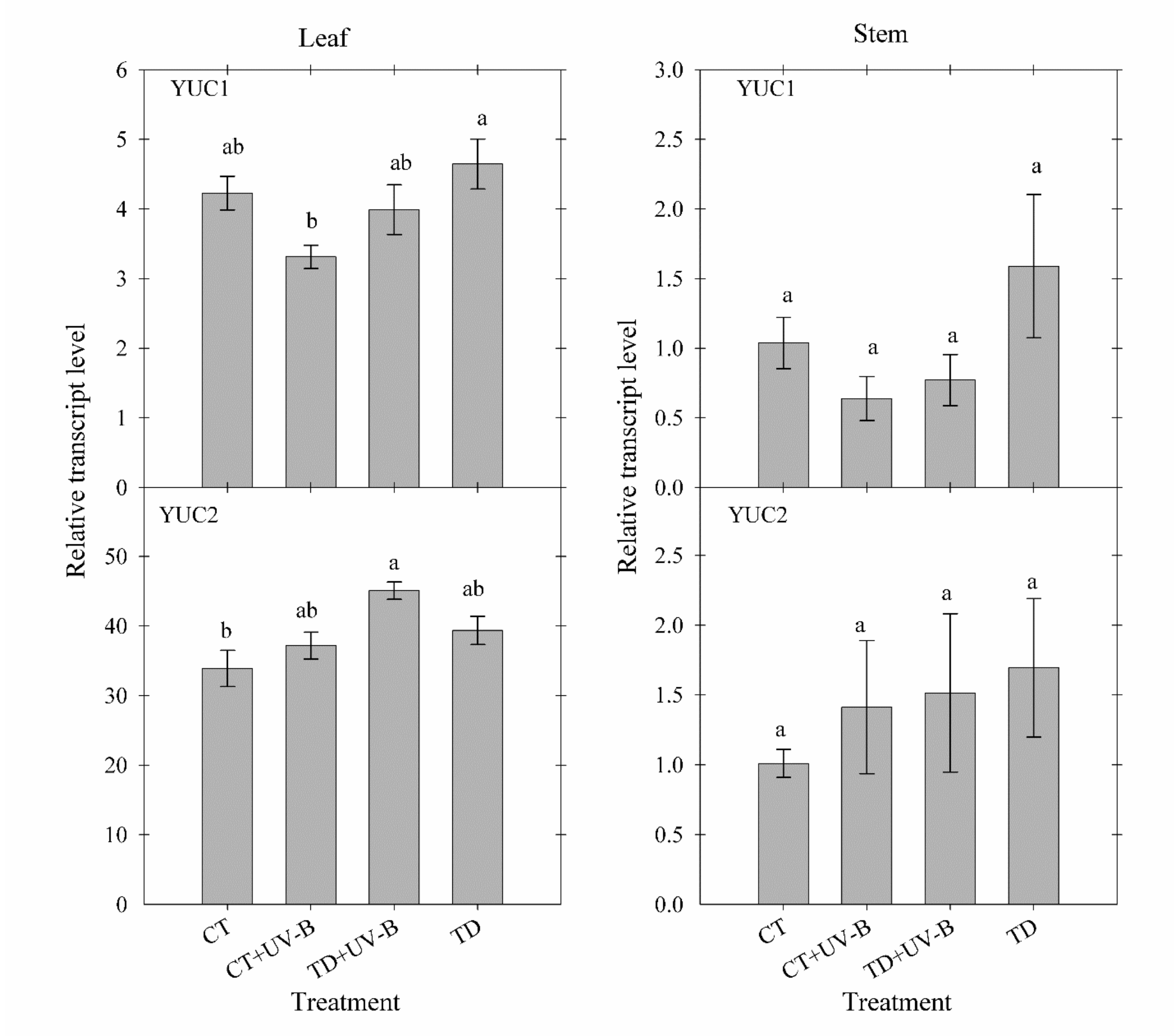

Fig. 9. Effect of 6 h daily UV-B treatment (+UV-B) in the middle of the light period for 10 days on transcript levels of the *YUC1* and *YUC2* in pea plants under two temperature regimes (constant temperature (CT = 20ºC); and a 6 h daily temperature drop (TD = 21→13ºC)). Both UV-B and temperature drop were applied in the middle of the light period. The transcript levels, normalized against α-tubulin, are shown as mean ± SE of fold change relative to CT in apical stem tissue. Three repeated samples, each consisting of 6 plants, were analyzed in each case. Different letters indicate significant difference at $p \leq 0.05$ (One-way ANOVA followed by Tukey`s test).

Table 1. The genes, their Genebank accession numbers, the primers and the probes (TAMRA; Applied biosystems) used for the qRT-PCR analysis of transcript levels of GA metabolism genes in pea plants.

Gene	Primer and Probe sequences (5` to 3`)	(Gen-Bank accession number)
α-Tubulin	Fw: TGAGGGAGTGCATTTCGATTC	U12589
	Rw: AGCTCCCAGCAGGCGTTT	
	P: CATCGGTCAAGCCGGTAT CCGGGTA	
GA2ox1	Fw: CATAGCTCCTTCTTTATCAATGTTGGT	AF056935
	Rw: TGCCATTTGCCAAAACTCTATGT	
	P: ACTTTTGAACCTCCCATTAGTCATAACCTGAAGA	
GA2ox2	Fw: GGT TGA TAA GCC CGT TAT CGA A	AF100954
	Rw: GGC CCA TGT AAA GGG CCT ATA T	
	P: TGG TGA CGG CCC ATA GCC CAT G	
GA20ox1	Fw: CAT TCC ATT AGG CCA AAT TTC AAT	U70471
	Rw: TGC CCT ATG TAA ACA ACT CTT GTA TCT C	
	P. CAA TAT TGG TGA CAC CTT CAT GGC TCT TTC A	
GA3ox1	Fw: TTC GAG AAC TCT GGC CTC AAG	AF001219
	Rw: ATG TTC CTG CTA ACT TTT TCA TGG	
	P: TCA TCA TAT TGC ACG ACA ATA TCA CAG AAT CTG G	

Na	Fw: CTT AAT CAT GGA GTT AGA GCT ATG CAA	AF537321
	Rw: TTC CTA GCC TTG AGC GCT TTA	
	P: TCA ATG TTC CTG GAT TTG CAT ACT	
Ls	Fw: TTA TTT GAA CAT ATT TGG GTG GTT GA	AY245442
	Rw: CAA TCT TTG ATC TCA TGT CGA AAA A	
	P: CGT CTC GAA CGC CTT GGA ATA TCT CGA	
Lh	Fw: TGG ATA AGC AAC TTG TGG GAA AA	U63652
	Rw: CCG CTT GGG CAT ATT TCT CAT	
	P: CCA GAC CAG TGG ATC CCA GAG AGA TTT CTT	

Key: Fw, forward primer; Rw, reverse primer; P, probe sequence (TAMRA; Applied Biosystem)

Table 2. The genes, their Genebank accession numbers, and the primers sequences used for the qRT-PCR analysis of transcript levels of *YUCCA* genes in pea plants.

Gene (GenBank accession number)	Primer sequences (5` to 3`)
YUC1 (HQ439907.1)	Fw: GGTGATGGAAGGTGTGAAGG
	Rw: AGCCAACTAGGCACATTGCT
YUC2 (HQ439908.1)	Fw: ACGATCGGTTACGTCTCCAC
	Rw: CGAATTCGGCAT CATTTTTCACT

Key: Fw, forward primer; Rw, reverse primer

Table 3. Impact of 6 h daily UV-B exposure (+UV-B; -UV-B: control plants not exposed to UV-B) in the middle in the light period for 10 days on morphology of pea plants at two different temperature regimes (constant temperature (20℃) and during a 6 h daily temperature drop (21 →13℃; same daily mean temperature as CT)). Results are mean ± SE of 6 individual plants in each of three repeated experiments (n=18). Different letters in a column indicate significant differences at $p \leq 0.05$ (One-way ANOVA followed by Tukey`s test).

Temperature	UV	Internode length (cm)	Number of leaves	Total leaf area (cm^2)	Specific leaf area ($cm^2\ g^{-1}$)
Constant temperature (20℃)	-UV-B	4.9±0.1a	5.0±0.0a	81.1±3.1a	700.2±49.8a
	+UV-B	4.6±0.1a	4.7±0.2a	52.6±4.6c	577.1±97.9a
Temperature drop (21→13℃)	-UV-B	4.6±0.0a	4.7±0.2a	74.7±5.7ab	681.2±13.5a
	+UV-B	3.4±0.1b	4.3±0.2a	61.09±2.2bc	634.6±62.8a

Paper - II

Amsalu Gobena Roro, Tone Ingeborg Melby, YeonKyeong Lee, Line Nybakken, Knut Asbjørn Solhaug, Sissel Torre, and Jorunn E. Olsen

UV-B signaling in pea involves LONG1 and LIP1, homologs of *Arabidopsis* HY5 and COP1

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Abstract

In *Arabidopsis thaliana*, COP1 and HY5 are central players in UV-B signaling resulting in formation of UV-B-protecting compounds and altered morphogenesis. However, information about UV-B signaling in other species is limited. Compact morphology under UV-B is thought to contribute to lower susceptibility to UV-B by reducing UV-B interception. In pea, we have demonstrated a UV-B-induced reduction in contents of bioactive GA_1 in shoot tips and young leaves. However, whether GA levels, and thus degree of extension growth, affect the susceptibility to UV-B, are unclear. We here aimed to investigate the roles of the HY5 and COP1 homologs LONG1 and LIP1 in pea in protection towards UV-B-related damage and altered morphogenesis under UV-B as well as the effect of GA in these responses. Consistent with LONG1 and LIP1 as UV-B signaling compounds in pea, the *long1* and *lip1* mutants

exhibited hypersensitivity and higher resistance to UV-B compared to the WT, respectively, probably due to their lower and higher levels of specific flavonoid glycosides. The dwarfed *le* GA biosynthesis mutant and the elongated *la cry-s* GA signaling mutant, which behaves like being GA saturated, were both more resistant to UV-B-related damage than the WT, probably due to higher levels of specific flavonoid glycosides, as shown in *le*. GA_3 application did not affect the sensitivity to UV-B-related damage. The *long1*, *cry-s* and *le* mutants did not exhibit UV-B reduction in elongation growth, except a slight height reduction in the *le* mutant when UV-B was combined with temperature drop. The *lip1* mutant behaved similar to the WT. These studies demonstrate that LONG1 and LIP are essential UV-B signaling components in pea, and that GA content and degree of extension growth do not affect susceptibility to UV-B-related damage. However, ability to adjust the GA levels and GA response is required for UV-B-induced reduction of elongation growth.

Key words: Flavonoids, Gibberellin, LIP1, LONG1, *Pisum sativum*, UV-B, UV-B signaling

1. Introduction

Plants are well known to modify their molecular and biochemical processes as well as their morphology to adjust themselves to environmental conditions like UV-B radiation, high irradiance and low temperature (Havaux & Kloppstech, 2001; Santos *et al.*, 2004). The UV spectrum is divided into three regions depending on the wavelengths and energy; UV-C (≤ 280 nm), UV-B (280 -315 nm) and UV-A (315- 350 nm), of which UV-C and UV-B are the most energetic (Rozema *et al.*, 1997). However, all the shortest wavelengths including the UV-C region and most of the shortest wavelength region of UV-B, are filtered out by the atmospheric ozone before it reaches the earth's surface, while solar UV-A passes almost unaltered through the atmosphere. At natural conditions, the levels of UV-A and UV-B radiation reaching the ground surface, are affected by different factors including altitude, latitude, season, cloud pattern and time of the day (Madronich *et al.*, 1998; McKenzie *et al.*, 2001).

Plant responses to UV-B and UV-A vary between plant species and background light quality, and negative effects of UV-B on DNA can be ameliorated by a UV-A and blue light-activated DNA repair mechanism through the enzyme photolyase (Wilson *et al.*, 2001; Ibdah *et al.*, 2002). UV-B radiation is also well known to induce the biosynthesis of UV-B protecting phenolic compounds such as flavonoids, which act as shielding components and antioxidants in the defense against high levels of reactive oxygen species (ROS), formed among others in response to UV radiation (Day, 1993; Dai *et al.*, 1997; Jenkins, 2009).

Although high UV-B levels may cause general stress responses, plants are generally well protected towards UV-B in nature. However, some cultivated species or cultivars may be more sensitive towards UV-B due to breeding which has reduced the contents of UV-protecting

compounds such as flavonoids (Barnes *et al.*, 1988; Li *et al.*, 1993; Jenkins, 2009). There are also differences in the sensitivity to UV-B among different plant species. It has been reported that plants distributed along low latitudes or high altitudes where UV-B levels are the highest, have more pronounced adaptive mechanisms than plants from higher latitudes or lower elevations (Sullivan *et al.*, 1992; Turunen & Latola, 2005). Moreover, variability in UV-B sensitivity between different crop species has been reported (Barnes *et al.*, 1990; Dai *et al.*, 1994)

Light-induced changes in plant growth and development are complex and known to be regulated through multiple pathways. Plants have a range of photoreceptors perceiving different parts of the light spectrum; the red and far-red-light perceiving phytochrome system, the blue light UV-A- perceiving cryptochromes, phototropins and Zeitlupe family members as well as the UV-B-sensor UVR8 (UV RESISTANCE LOCUS 8), which so far has only been identified in *Arabidopsis thaliana* (Kliebenstein *et al.*, 2002; Brown & Jenkins, 2008; Rizzini *et al.*, 2011). A range of reports has indicated that photoreceptor localization is mainly affected by the light quantity and quality (Nagy *et al.*, 2000; Kircher *et al.*, 2002; Bauer *et al.*, 2004). In dark-grown seedlings of *A. thaliana*, phytochrome A (phyA) is localized in the cytosol, whereas phy B to phy E are predominantly localized in the cell compartment. Similarly, it was observed that in *A. thaliana*, the UVR8 protein is localized in the cytoplasm as well as the nucleus, and that UV-B irradiation enhances its accumulation in the nucleus (Brown *et al.*, 2005; Kaiserli & Jenkins, 2007).

In the nucleus the dimeric UVR8 forms monomers in response to UV-B, and the monomer then interacts with CONSTITUTVELY PHOTOMORPHOGENIC 1 (COP1) (Favory *et al.*, 2009; Rizzini *et al.*, 2011; Cloix *et al.*, 2012; Jenkins, 2014). COP1 appears to affect the same genes

as UV-B, indicating that UVR8 and COP1 act together in UV-B responses (Favory *et al.*, 2009). In *A. thaliana* COP1 has been shown to be part of an E3 ubiquitin ligase complex, which in dark-grown seedlings targets positive regulators of photomorphogenesis like the transcription factors ELONGATED HYPOCOTYL 5 (HY5) and HY5 HOMOLOGUE (HY5) for destruction (Osterlund *et al.*, 2000; Lau & Deng, 2012). Opposite to this, in response to UV-B, COP1 promotes expression of *HY5* in addition to a wide range of other genes (Oravecz *et al.*, 2006). Thus, although COP1 has been shown to act as a repressor of photomorphogenesis, it apparently acts positively in UV-B-related photomorphogenesis (Lau & Deng, 2012; Jenkins, 2014). This may be associated with inactivation of the E3 ubiquitin ligase activity of COP1 upon interaction of COP1 with UVR8, by which HY5 is stabilized and protected from degradation under UV-B (Huang *et al.*, 2012). On the other hand, as mentioned above, when UV-B is not present, the UVR8-COP1 complex is not formed and COP1 targets HY5 for degradation. However, the mode of action of UV-B on HY5 appears to be more complex since more degradation of HY5 was observed in *cop1* mutants than in the wild type (WT) after UV-exposure (Jenkins, 2014). Furthermore, HY5 has been shown to stimulate expression of *COP1* by binding to its promoter (Huang *et al.*, 2012).

Flavonoids, which accumulate in the epidermal and sub-epidermal cell layers, act in protection against UV-B, and plants lacking flavonoids are thus highly UV-B sensitive (Li *et al.*, 1993; Landry *et al.*, 1995; Jansen *et al.*, 1998; Tilbrook *et al.*, 2013). HY5 is known to stimulate expression of genes involved in the production of flavonoids (Brown *et al.*, 2005; Tilbrook *et al.*, 2013). Consistent with this, *hy5* mutant plants are hypersensitive to UV-B (Brown *et al.*, 2005; Brown & Jenkins, 2008). Flavonoid biosynthesis is largely regulated at the transcriptional level, and UV-B has been shown to promote expression of the *CHALCONE SYNTHASE* (*CHS*) and *FLAVONOL SYNTHASE* (*FLS*) genes, which are involved in the

biosynthesis of all flavonoids and the specific flavonoid group denoted flavonols, respectively (Lepiniec *et al.*, 2006; Jenkins, 2008; Stracke *et al.*, 2010).

In visible light-related photomorphogenesis HY5 apparently acts in light signaling through crosstalk with plant hormones (Alabadi *et al.*, 2008; Chen *et al.*, 2008; Weller *et al.*, 2009; Li & Huang, 2011a). Elongated hypocotyls and lateral roots in *hy5* mutants also support the involvement of hormone-related genes in the HY5 regulatory network (Li & Huang, 2011b; Li *et al.*, 2012). Homologs of *COP1* and *HY5* genes, denoted *LIP1* and *LONG1*, respectively, have been described in pea, and LONG1 was shown to somehow be involved in up-regulation of the GA inactivation gene *GA2-oxidase 2* (*GA2ox2)* after transfer of dark-germinated seedlings to light (Weller *et al.*, 2009).

In the greenhouse industry control of shoot elongation of ornamental plants and transplants of vegetables is essential since more compact plants occupy less space in the greenhouse, are easier to handle and transport and might have higher ornamental value. To obtain compact plants, chemical growth regulators are commonly used, but due to their potentially negative effects on human health and the environment (De Castro *et al.*, 2004; Sørensen *et al.*, 2009), alternative methods are highly interesting. In temperate areas daily temperature drop treatments or lower day than night temperature are commonly used to control shoot elongation since such conditions can be obtained by opening the greenhouse vents (reviewed in Myster & Moe, 1995). In pea such treatments decrease the level of bioactive GA_1 through enhanced inactivation due to increased *GA2ox2* expression (Grindal *et al.*, 1998; Stavang *et al.*, 2005; Stavang *et al.*, 2007; Stavang *et al.*, 2010). However, during warmer periods or in warmer areas such treatments are not feasible without cooling systems. Since non-damaging levels of UV-B are well known to reduce elongation growth (as discussed above), exploiting this response,

possibly in combination with a daily temperature drop treatment, might be interesting as a tool to control shoot elongation in greenhouses. However, since high levels of UV-B might result in damage such as DNA-damage to plants (e.g. Hollosy, 2002), great care must be taken to avoid this.

GAs are well known to control shoot elongation in plants and has been shown to act through inhibition of DELLA inhibitors (Yamaguchi, 2008). In pea reduced shoot elongation and leaf area under non-damaging levels of UV-B was shown to be associated with reduced levels of the bioactive GA_1, apparently as a consequence of reduced inactivation and possible reduced biosynthesis (Roro *et al.*, Paper I). Although the susceptibility of *hy5* mutants to UV-B-related damage was ascribed to reduced flavonoid contents (Brown *et al.*, 2005; Brown & Jenkins, 2008), it might also be hypothesized that their elongated phenotype due to high levels of bioactive GA, as shown in pea (Weller *et al.*, 2009), adds to the UV-B susceptibility.

To our knowledge, involvement of HY5 and COP1 in UV-B signalling has so far been demonstrated in *A. thaliana* and information in other species is scarce. Thus, to extend the knowledge on UV-B signaling to other plants than *A. thaliana*, we aimed to evaluate the involvement of the HY5 and COP1-homologs in pea, LONG and LIP1, respectively, in UV-B responses in pea. To shed light on this, effects of UV-B on shoot elongation, flavonoid contents and DNA damage in pea plants mutated in LONG1 and LIP1, as compared to the WT, were studied. Furthermore, to test the hypothesis that high GA levels might make plants more susceptible to UV-B-related damage, we also studied effects of mutation in the pea GA biosynthesis gene *LE* (Lester *et al.*, 1997), application of GA_3, and mutation in the two GA signaling *DELLA* genes described in pea, *LA* and *CRY*, which act as negative regulators of GA action (Weston *et al.*, 2008). Since a combination of UV-B and a daily temperature drop

treatment might potentially be interesting as a tool to control shoot elongation in greenhouses, the effects of UV-B on shoot elongation under constant temperature and under a daily temperature drop exposure were studied.

2. Materials and methods

2.1. Plant materials and pre-growing conditions

Seeds of wild-type of pea (*Pisum sativum* L., cv Torsdag) and the pea mutants, *long1*, *lip1*, *le* and *la cry-s* were sown in 11 cm pots containing a standard fertilized sphagnum peat (Tjerbo Torvabrikk, Rakkestad, Norway) and perlite (3:1 w/w). The pre-treatment cultivation was done in growth chambers (75 x 80 x 80 cm; manufactured by Norwegian University of Life Sciences). During the pre-cultivation period the growth conditions were adjusted to a constant temperature of 20℃, a 12 h photoperiod, a photosynthetic photon flux density (PPFD) of 100 µ mol m^{-2} s^{-1} supplied from fluorescent tubes (MASTER TL-D Super 80 36W/840 Philips, Eindhoven, The Netherlands) and a red: far-red (R:FR) ratio of 1.7, achieved through addition of incandescent lamps (Osram, Munich, Germany). Irradiance was measured with a quantum sensor (Model L1-185 quantum sensor; Li-COR, Lincoln, NE, USA). The air humidity in these chambers could not be precisely controlled, but trays with water were placed beneath the bottom plates of the chambers, according to Stavang *et al.*, (2005). The pre-treatment cultivation ended at day 6 after sowing when the plant had 1-1.5 cm long shoots.

2.2. Experimental conditions

At day six after sowing, plants were transferred to different treatments: 1) constant temperature (CT) at 20℃ and 2) a 6 h UV-B treatment in the middle of the 12 h light period under CT 3) a so-called temperature drop treatment (TD) where plants were grown at 21℃ except for 6 h in the middle of the light period when temperature was reduced from 21℃ to 13℃ for 6 h and 4) a 6 h UV-B treatment provided in the middle of the 12 h light period together with the TD treatment. The daily mean temperature was 20℃ in all cases and all other conditions and the growth chambers were as described above. Two or three UV-B fluorescent tubes (UVB-313, Q-Panel Co., Cleveland, OH, USA) were used. A 0.13 mm thick cellulose diacetate foil (Jürgen Rachow, Hamburg, Germany) put 10 cm under the UV-B lamps, was used to filter the shortest part of the UV-wavelengths, i.e. wavelengths below 290 nm. Since the chamber walls were non-reflecting with respect to UV-B, to obtain more even UV-B distribution in the chambers, the inner chamber walls were laminated with aluminum foil before the start of the UV-B treatment. The fluence rate of UV-B was measured at the start of the experiments with a broadband UV-B sensor (SKU340, Skye Instruments, Powys, UK) at all sides of a tetrahedron at the top of the plant canopy in the middle of each chamber according to (Björn, 1995). Simultaneous measurement with this broadband UV-B sensor and an Optronic model 756 spectroradiometer (Optronic laboratories, Orlando, FL, USA) generated a calibration factor, which was used for calculation of the absolute UV-B irradiation. In different experiments absolute UV-B was approximately 0.25, 0.35 or 0.5 W m^{-2} about 15 cm above the chamber floor (see figure legends for details on UV-B levels). The efficiency of the UV-B tubes was reduced by 25% under the temperature drop compared to under constant temperature, so the UV-B levels could not be directly compared under CT and TD. In experiments with genotypes of different heights, stacks of empty pots were put under the shortest plants and the height of

the pot stacks were adjusted as the plants grew, to ensure the shoot apices were at the same height. The experiments were performed twice. Plant height of 10-15 plants per genotype was recorded in each of two experiments.

2.3. Application of GA_3

To evaluate if exogenously applied gibberellic acid (GA_3) affect the susceptibility to UV-B related damage, GA_3 (Sigma-Aldrich, St. Louis, MO, USA) was applied to first unfolded leaf. On the second day of 10 days of UV-B exposure at 0.35 W m^{-2}, when the first leaf had unfolded, 10 µg GA_3 in 10 µl 96% ethanol or 10 µl 96% ethanol only (mock treatment) were applied to each of ten plants. Control plants that did not receive GA_3 or ethanol under the UV-B exposure were included for comparison

2.4. Analysis of flavonoids by HPLC

After 10 days of UV-B exposure, the lamina of the third leaf above the soil was collected from each of 10 plants per genotype. Such leaf materials from 10 plants per genotype were also collected from control plants not exposed to UV-B. The leaves were dried in a drying cabinet at 30°C for two days and then the middle veins and petioles were removed with a scalpel. The leaf materials were then weighed on a micro-scale (Mettler Toledo, Oslo, Norway) and the leaf material from individuals plant transferred to individual Precellys vials, each containing one stainless steel bead of 5 mm diameter. After addition of 600 µl methanol (MeOH) to each vial, the samples were homogenized on a Precellys 24 homogenizer (Bertin Technologies,

Montigny-le-Bretonneux, France) for 30 sec at 6500 rpm. The samples were then placed in an ice bath for 15 min, homogenized again for 15 sec, centrifuged at 15,000 rpm for 3 min and the supernatant of each sample poured into a clean glass tube. The residue was added 600 µl MeOH, homogenized for 30 sec and again centrifuged. The last procedure was repeated three times, and the residue was then colorless.

The methanol was evaporated from the test tubes with a vacuum concentrator (Eppendorf AG, Hamburg, Germany) at 30℃ for 1 h, and the dried extracts stored in the freezer (-20°C) until HPLC analysis. The phenolic compounds were analyzed on an Agilent HPLC (Agilent, Series 1100, Germany), consisting of a binary pump (G1312A), a thermostated autosampler (G1329A), a thermostated column oven (G1316A) and a diode array detector (G1315B). The phenolic metabolites were separated using an ODS Hypersil C18 (4.6 × 50 mm) HPLC column (Thermo Scientific, Waltham, Massachusetts, USA). The samples were re-dissolved in 400 µl methanol : water (1:1) and eluted (flow rate 2 ml min-1) using a methanol : water gradient (Julkunen-Tiitto and Sorsa 2001). The auto injection volume was 20 µl, and all runs were performed at +30 ºC. The phenolic metabolites were identified by comparing their retention times and UV spectrum with those of commercial standards.

2.5. Extraction of DNA

After 10 days under the different treatments, the lamina of leaf number 3 as counted from the basis of the plant, was harvested into liquid nitrogen from each of 5 plants per treatment and genotype and kept in darkness at -80°C until analysis. The leaf materials were homogenized by a Mixer Mill MM 301 (Retsch, Germany) and a DNeasy Plant Mini Kit (cat no 69104,

Qiagen GmbH, Germany) was used to extract total DNA. DNA extraction took place under dim yellow light (Strand Filters, number 401 Yellow, Strand Lightning Ltd, UK) to avoid uncontrolled photorepair. The DNA concentration was determined using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Inc., USA). Thereafter the samples were stored at -20°C for some days until analysis of DNA damage.

2.6. Assay of DNA damage

Cyclobutane pyrimidine dimers (CPDs) were quantified by enzyme-linked immunosorbent assay (ELISA) using OxiSelect™ UV-Induced DNA damage ELISA Kit for CPD Quantification (Cell Biolabs, Inc., USA) according to the manufacturer`s instructions. DNA samples were diluted to 2 µg ml^{-1} or less in cold phosphate-buffered saline (PBS) before converted to single-stranded DNA. According to the assay protocol, the absorbance of the reaction mixture was measured on a microplate reader (Biochrom Asys UVM 340 with KIM, UK) using 450 nm as the primary wave length. For each treatment and genotype five biological repeats (plants) were analyzed.

2.7.Statistical Analysis

To test for effects of the different treatments, one way analysis of variance (ANOVA) was performed using a completely randomized design using (Minitab software versions 16.1.1, State College, Pennsylvania, USA), followed by Tukey's test. Differences with $p \leq 0.05$ were considered significantly different.

3.0. **Results and discussion**

Roles of COP1 and HY5 in UV-B signaling related to formation of UV-B protecting compounds and UV-B-related photomorphogenesis have to our knowledge hitherto only been demonstrated in the rosette plant *A. thaliana*. Our study extends the knowledge on UV-B signaling to a species showing an upright growth habit due to internode elongation, by demonstrating roles of the HY5 and COP1 homologs in pea, LONG1 and LIP1, in UV-B control of shoot elongation and production of UV-B-protecting flavonoids. In addition, since LONG1 is known also to act in light-GA cross-talk in pea (Weller *et al.*, 2009), and GA levels were shown to decrease in response to UV-B exposure in pea plants (Roro *et al.*, Paper I), the impact of mutation in GA biosynthesis and GA signaling in the UV-B responses was evaluated. We show here that adjustment of the GA levels or the GA response is required for UV-B-induced reduction in elongation growth, but that susceptibility to UV-B-related damage is apparently not affected by GA levels or GA response and degree of shoot elongation.

3.1. The long1 and *lip1 mutants show higher and lower degree of UV-B related damage than the WT*

Under high, stressful UV-B levels leaf curling is a well-known UV-B photomorphogenic response that reduces the leaf area (Greenberg *et al.*, 1997; Jansen *et al.*, 1998). Generally, stressful, high levels of UV-B radiation are accompanied by strongly increased production of reactive oxygen species (ROS), which in turn can result in damage to biomolecules like DNA, proteins and membranes (Hollosy, 2002). Among the most common DNA damage products are cyclobutane pyrimidine dimers (CPD) (e.g. Britt, 1995; Hollosy, 2002; Li *et al.*, 2002; Lo

et al., 2005). In this study we evaluated UV-B induced DNA damage in different pea genotypes by measuring the level of CPD.

Pre-experiments with different levels and durations of UV-B showed that the *long1* mutant was highly vulnerable to UV-B-related damage. Even after a 2 h daily exposure to 0.5 W m^{-2} UV-B for 10 days under a photosynthetic active radiation (PAR) of 100 µmol m^{-2} s^{-1} (same PAR in all experiments, and UV-B always provided in the middle of the light period), where the WT showed slight leaf curling only, the *long1* mutant exhibited severe damage with leaf rolling, chlorotic and necrotic spots, twisted tip and brittle stem. A UV-B level/duration that resulted in quite severe leaf curling in the WT was lethal to the *long1* mutant, like 0.5 W m^{-2} for 6 h daily. Exposure to 30 min of about 0.25 W m^{-2} (Fig. 1) or 0.35 W m^{-2} (data not shown) UV-B, resulted in a little or no leaf curling in the WT, but considerably more leaf curling in the *long1* mutant (Fig. 1). More UV-B-related visible damage in the *long1* mutant than the WT was associated with more than 5-fold higher CPD-level in the *long1* mutant (analysed in 3rd leaf from basis) after 30 min daily exposure to 0.25 W m^{-2} in the middle of the light period for 10 days (Fig. 2a). This is in accordance with the earlier reported higher susceptibility to UV-B of the *hy5* mutant than in the WT plants in *A. thaliana* (Brown *et al.*, 2005; Brown & Jenkins, 2008).

Smaller or larger degree of leaf curling/damage was observed in the WT UV-B in the experiments described here where 25-50 W m^{-2} UV-B was used in chambers with UV-B reflecting walls (as measured from different sides according to (Björn, 1995), using a “flat” sensor). However, in an earlier study of pea where the chamber walls were not UV-B reflecting and the absolute UV-B level (as measured from above), was 0.45 W m^{-2}, visible damage was not observed in the WT (Roro *et al.*, Paper I). In this former experiment, plants were pre-grown

in a greenhouse compartment (Ås, Norway, 59°39'47''N 10°47'38''E) in May-July 2011, when irradiance from natural light is normally high. Thus, the higher PPFD during pre-growth might have contributed to make these plants more UV-B tolerant than in the present experiments where plants were pre-grown in the growth chambers at a PPFD of 100 µmol m^{-2}. More damage when the plants were exposed to UV-B from all sides due to UV-B reflection from the chamber walls could probably also be ascribed to considerably lower UV-B screening in the lower than the upper leaf surface, and thus deeper penetration of UV-B into the lower leaf surface. In *Vicia faba* leaves UV-B screening was shown to be 2-4 fold higher in the upper than the lower leaf surface (Markstädter *et al.*, 2001). In experiments where the *long1* mutant was pre-grown in a greenhouse compartment in May-July 2011, and thereafter exposed to 0.45 W m^{-2} in growth chambers with UV-B non-reflecting walls, the *long1* mutant exhibited leaf curling/damage in contrast to the WT (results not shown for *long1*, WT results in Roro *et al.*, Paper I). This supports the hypersensitivity of the *long1* mutant to UV-B under conditions where the WT was not visibly damaged.

Compared to the WT, the *lip1* mutant in pea showed considerably less leaf curling in response to 6 h daily U-B exposure at 0.25 W m^{-2} (Fig. 1) and 0.5 W m^{-2} (data not shown) in the middle of the light period for 10 days. Less damage was confirmed by the significantly lower CPD levels in the *lip1* mutant (about 60%; 3rd leaf from basis) than the WT after the UV-B exposure at 25 W m^{-2}. Higher UV-B resistance in the *lip1* mutant in pea than in the WT is opposite to the situation reported for the *cop1-4* mutant in *A. thaliana* (Oravecz *et al.*, 2006). Thus, it appears that the pea COP1 homolog LIP1 acts differently from COP1 with respect to a role in signaling leading to protection towards UV-B related damage. In *A. thaliana* it has been reported that HY5 is stabilized and protected from degradation when COP1 interacts with UVR8 since the E3 ubiquitin ligase activity of COP1 is then inactivated (Huang *et al.*, 2012).

Without this E3 ubiquitin ligase activity of COP1, HY5 is not targeted for destruction, and thus stabilized. I then follows that when UV-B is not present, the UVR8-COP1 complex is not formed and COP1 targets HY5 for degradation. However, the situation appears more complex, since more degradation of HY5 was observed in the *A. thaliana cop1* mutants than in the wild type (WT) after UV-exposure (Jenkins, 2014). If LIP1 in pea acts through the pea HY5 homolog LONG1 in UV-B responses, higher UV-B resistance in the *lip1* mutant is consistent with a situation with higher levels of the HY5 homolog LONG1 in the *lip1* mutant. Although this remains to be quantified with respect to UV-B-signaling, the previous study of visible light-related photomorphogenesis has demonstrated genetic interaction between LONG1 and LIP1 (Weller *et al.*, 2009). Thus, the involvement of LIP1 in UV-B signaling seems to resemble the situation described for visible-light-related photomorphogenesis in *A. thaliana* and pea (Osterlund *et al.*, 2002; Weller et al. 2009).

3.2. The le GA biosynthesis mutant and la cry-s GA signaling mutant show less UV-B related damage than the WT

In addition to showing lower degree of UV-B related damage than the WT (Fig. 1-2), the *lip1* mutant is dwarfed like the *A. thaliana cop1* mutant. In accordance with being dwarfed, the *lip1* mutant was shown to contain lower levels of bioactive GA_1 than the WT (Weller *et al.*, 2009). In addition to the higher degree of UV-B related damage shown here in the *long1* mutant (Fig. 1-2), the *long1* mutant is elongated like the *hy5 A. thaliana* mutant. The *long1* mutant was shown to contain higher levels of bioactive GA_1 than the WT (Weller *et al.*, 2009). Furthermore, treatment with the GA biosynthesis inhibitor paclobutrazol was reported to enhance tolerance to elevated UV-B levels, at least with respect to photosynthesis efficiency in soybean (*Glycine max*) (Kraus *et al.*, 1995). Thus, we asked the question whether high level

of bioactive GA, and accordingly high degree of shoot elongation, might make plants more susceptible to UV-B-related damage. In accordance with such an idea, compared to the WT, the *le* mutant showed less visible damage in response to 6 h daily UV-B exposure at 0.25 W m^{-2} (Fig. 1) and 0.35 W m^{-2} UVB (data not shown) for 10 days. After the 0.25 W m^{-2} UV-B treatment, the *le* mutant showed about 53% lower CPD level in the 3rd leaf from basis than the WT (Fig. 2).

If it is true that low GA levels make plants more resistant to UV-B-related damage, it might be expected that an elongated GA signaling mutant, showing a saturated GA response independently of environmental conditions, would be more susceptible to UV-related damage. To test this hypothesis we investigated the effect of UV-B on visible damage and CPD levels in the *la cry-s* mutant of pea, which is mutated in the two *DELLA* inhibitor genes (*LA* and *CRY*) described in pea (Weston *et al.*, 2008). However, this mutant did not exhibit any visible damage or leaf curling in response to the 10 days daily 30 min UV-B exposure at 0.25 W m^{-2}, or no or only slight leaf curling (less than the WT) in response to the 6 h daily exposure at this UV-B level (Fig. 1). Correspondingly, after the 6 h daily UV-B treatment the *la cry-s* mutant showed significantly lower CPD levels (80% less at 6 h UV-B exposure) compared to the WT (Fig 2). Accordingly, the saturated GA response in the elongated, slender *la cry-s* GA signaling mutant does not increase the susceptibility to UV-B-related damage, but instead increases the UV-B resistance.

3.3. Application of GA_3 does not result in higher susceptibility to UV-B-related damage

To further shed light on the effect of GA on susceptibility to UV-B-related damage, GA_3 was applied to the lamina of the first leaf from the basis of WT plants exposed to UV-B at 0.35 W

m^{-2}. As reported earlier (Roro *et al.*, Paper I), GA_3 application counteracted the inhibitory effect of UV-B on shoot elongation (data not shown). However, after 10 days of the UV-B exposure no difference in leaf damage was observed between plants with a GA_3-applied leaf, mock treatment with ethanol only or unapplied leaves (Fig. 3). Analysis of CPDs in the 3rd leaf from the basis did also not reveal any significant effect of the GA_3 treatment on the levels of this UV-B related DNA damage product (Fig. 2). Although GA_3 undoubtedly reached the apex and enhanced shoot elongation under UV-B, it might be questioned how much of the GA_3 that reached the 3rd leaf. However, that the leaf curling was similar in all leaves (as shown for leaf 1, 3 and 4 in Fig. 3), including the youngest ones (data not shown), which are known to be strong sinks, irrespective of GA_3 application, mock treatment (ethanol only) or no application, indicate that susceptibility to DNA-related damage is not dependent on GA content.

3.4. Lower and higher sensitivity to UV-B-related damage correspond with higher and lower levels of specific flavonoids

Flavonoids and related phenolic compounds absorb strongly in the UV region of the spectrum, and cultivars and genotypes with high levels of such compounds are better protected against damaging effects of UV-B radiation than plants with lower levels (Murali & Teramura, 1986; Cen & Bornman, 1993; Ormrod *et al.*, 1995; Gonzalez *et al.*, 1996; Caasi-Lit *et al.*, 1997). Consistent with this, mutants lacking UV-protecting components are highly sensitive to ambient levels of UV-B radiation (Landry *et al.*, 1995). To further evaluate the roles of LONG1 and LIP1 as a signaling component in formation of UV-B protecting phenolic compounds in pea, the content of such compounds were analyzed by HPLC. Eighteen different phenolic compounds were detected (Table 1). These included different glycosides of the flavonols quercetin, kaempferol and myricetin as well as the flavones luteolin and apigenin. These were

previously shown to be major flavonoid compounds in pea and a wide range of plant species, e.g. vegetables like broccoli (*Brassica oleracea*), french bean (*Phaseolus vulgaris*) and broad bean (*Vicia faba*) (Justesen *et al.*, 1998; Sultana & Anwar, 2008). The different glycosides of a specific flavonoid followed very similar patterns in response to UV-B and with respect to differences between genotypes (data not shown) and were accordingly grouped.

In the WT, the amounts of the glycosides of the flavonols kaempferol and quercetin increased significantly by 135% and 520%, respectively, in response to a 30 min daily UV-B exposure (0.35 W m^{-2}) for 10 days, whereas there were no such significant differences in the *long1* mutant (Fig. 4). The generally lower level of total phenolic compounds in the *long1* mutant than in the WT and after UV-B exposure, compared to unexposed control plants, can to a large extent be explained by the patterns of the glycosides of the flavones apigenin and luteolin, which were present in the highest amounts of the five recorded groups of flavonoid glycosides (Fig. 4). Although the contents of the glycosides of apigenin were generally about 70% lower in the *long1* mutant than in the WT, apigenin as well as luteolin glycosides decreased significantly in response to UV-B in the WT (65% and 69% decrease, respectively) as well as the *long1* mutant (62% and 78%, respectively) (Fig. 4). Thus, it is plausible that the hypersensitivity to UV-B related damage in the *long1* mutant (Fig. 1-2) is rather associated with its lack of induction of kaempferol and quercetin glycosides under UV-B. These observations are consistent with an important role of LONG1 in pea in the UV-B signaling leading to formation of these UV-B protecting compounds, similar to HY5 in *A. thaliana* (Jenkins, 2014).

In response to a 6 h daily UV-B treatment at 0.5 W m^{-2} for 10 days, the *lip1* mutant and the WT both showed a large increase in the levels of kaempferol glycosides, with close to 360%

increase in both cases, as compared to control plants not exposed to UV-B (Fig. 5). Also, under UV-B, the *lip1* mutant contained about twice the levels of kaempferol glycosides as the WT (Fig. 5). Furthermore, whereas no significant increase in quercitin glycosides was observed in the WT (only a trend of increase), the content of quercitin glycosides in the *lip1* mutant increased significantly by 155% in response to UV-B (Fig. 5). Also, the levels of quercetin glycosides were generally 6-7-fold higher in the *lip1* mutant than the WT, independently of UV-B-treatment or not. For myricetin glycosides the content was significantly higher in the *lip1* mutant (5-fold) as well as the WT (16-fold) after the 10 days of UV-B exposure, compared to their respective control plants (Fig. 5). However, the level of myricetin glycosides in the *lip1* mutant was 47% lower than that of the WT in the UV-B-exposed plants. Furthermore, apigenin glycoside levels were generally significantly higher in the *lip1* mutant than the WT, both in UV-B exposed (73%) and unexposed control plants (124%) (Fig. 5). However, no UV-B induction of the apigenin glycosides occurred, and whereas WT the level was unchanged, a 26 % decrease was observed under UV-B in the *lip1* mutant. The high content of apigenin glycosides, compared to the other phenolic compounds, could to a large extent explain the pattern of the total phenolic compounds (Fig. 5). Taken together, larger induction of kaempferol and quercetin glycosides in the *lip1* mutant than the WT under UV-B, might well be important for explaining the higher resistance towards UV-B-related damage of the *lip1* mutant compared to the WT (Fig. 1-2).

The high degree of resistance towards UV-B in the *lip1* mutant is consistent with a role of the COP1 pea homolog LIP1 in UV-B signaling in pea. However, whereas the *lip1* mutant is more resistant to UV-B-related damage and has higher levels of specific flavonoid glycosides than the WT (Fig. 1, 3, 5), the *cop1-4* mutant in *A. thaliana* is impaired in its UV-B tolerance and to a large extent blocked in its flavonoid accumulation (Oravecz *et al.*, 2006). Thus, although

the exact mechanisms by which COP1 acts in the UV-B response is still not well understood, in *A. thaliana* COP1 has been shown to act positively in UV-B-related photomorphogenesis by promoting expression of *HY5.* Also, interaction of COP1 with UVR8 results in inactivation of the E3 ubiquitin ligase activity of COP1, resulting in stabilization of HY5 (Oravecz *et al.*, 2006; Lau & Deng, 2012; Jenkins, 2014). On the other hand, in visible light-induced photomorphogenesis in *A. thaliana,* COP1 acts as a repressor due to its role in targeting HY5 for degradation in darkness (Osterlund *et al.*, 2000). As discussed above, the response of the *lip1* mutant of pea demonstrated here, is consistent with such a situation rather than a role of LIP1 as a positive regulator of UV-B-induced flavonoid production. The lack of LIP1 in pea may then result in stabilization of LONG1 and thus enhanced formation of flavonoids in response to UV-B. However, further studies of the action of LIP1 in pea are required to shed light on its mechanism of action in UV-B responses.

The *le* GA biosynthesis mutant, which was shown here to be less susceptible to UV-B-related damage than the WT (Fig. 1-2), showed significant induction (188% increase) of kaempferol glycosides in response to UV (Fig. 6). Although the contents of kaempferol glycosides increased significantly also in the WT (165% increase) under UV-B, the *le* mutant accumulated higher levels (117%) compared to the WT. Furthermore, the *le* mutant generally had significantly higher (17-fold and 5-fold for control and UV-B treated plants, respectively) levels of quercetin glycosides than the WT, although not significantly induced by UV-B in any case. The levels of the flavone glycosides (luteolin and apigenin) were also higher in the *le* muant than the WT (except apigenin glycosides in the control), but these decreased under UV-B. Thus, higher accumulation of flavonol glycosides is a plausible reason for higher UV-B-resistance in the *le* mutant compared to the WT.

Of the quantified flavonoid glycosides, kaempferol glycosides were consistently induced by UV-B in the more UV-B resistant *le* (Fig. 6) and *lip1* mutants (Fig. 5), i.e. to higher levels than in the less UV-B resistant WT, whereas there was no such induction in the UV-B-hypersensitive *long1* mutant (Fig. 4). The *lip1* and *le* mutants also had consistently higher levels of quercetin glycosides than the WT, and the *long1* mutant had lower. Collectively, these observations might indicate that the increased resistance to UV-B-related damage in the *lip1* and *le* mutants, and the hypersensitivity of the *long1* mutant, could be attributed to the contents of these specific flavonol glycosides. Although flavonoid contents in the *la cry-s* mutant awaits to be analysed, on basis of its higher resistance towards UV-B related damage (Fig. 1-2) it might be expected that this mutant also has higher levels of these flavonol glycosides than the WT.

3.5. Presence of LONG1 and adjustment of GA contents or response are required for UV-B-induced reduction in shoot extension

Like the *hy5* mutant in *A. thaliana* (Osterlund *et al.*, 2000), the *long1* mutant in pea is elongated (Weller *et al.*, 2009). When exposed to 0.35 W m^{-2} for 15 min, 30 min, 1 h or 1.5 h (data not shown) or 30 min of 0.25 W m^{-2} UV-B (Fig. 7), all in the middle of the 12 h light period (at a PAR of 100 µmol m^{-2} s^{-1}, like in all other experiments), the *long1* mutant did not show any significant difference in shoot elongation compared to when not exposed to UV-B. Also, the *long1* mutant did not respond to 30 min of such UV-B treatment when provided in the middle of a 6 h temperature drop (21°C to 13°C; otherwise 21°C; daily mean temperature 20°C as in the other experiments) given in the middle of the light period (Fig. 7). Also, consistent with our previous studies (Wendell *et al.*, unpublished) the temperature drop treatment alone did not

affect shoot elongation in this mutant (Fig. 7). The lack of reduction in shoot elongation in the *long1* mutant in response to UV-B and/or temperature drop is probably due its generally high GA_1 levels (Weller *et al.*, 2009), and inability to adjust these in response to these environmental factors. It was previously shown that after transfer of dark-germinated seedlings to light, this mutant does not exhibit normal reduction of shoot elongation. This was shown to be due to high GA_1 levels as a consequence of lack of up-regulation of the GA-inactivation gene *GA2ox2* in light (Weller *et al.*, 2009). The present results strongly support a role of LONG1 in UV-B signaling resulting in reduced elongation growth. When exposed to 4 h UV-B at 0.35 W m^{-2}, visible damage in the *long1* mutant was severe and elongation growth ceased due to damage of the shoot apex, and longer exposure was lethal (results not shown). This confirms the *long1* mutant`s hypersensitivity towards damaging effects of UV-B.

Although 30 min daily UV-B exposure at 0.25 W m^{-2} (Fig. 7) for 10 days did not affect shoot elongation in the WT significantly under constant temperature (20°C), when provided under a 6 h daily temperature drop (21°C to 13°C) in the middle of the light period, shoot elongation was reduced by 28% (Fig. 7). This was slightly more (9%) reduction compared to the inhibitory effect of temperature drop only. Although the UV-B levels during constant temperature and the temperature drop period are not directly comparable due to about 25% decrease in efficiency of the UV-B lamps under the temperature drop, this might suggest that the UV-B-response is affected by temperature regime. In an earlier study, where plants were exposed to UV-B from above only in a chamber with non-UV-B-reflecting walls, stronger inhibitory effect of 6 h UV-B under 6 h temperature drop (same temperature conditions as in the present study) was observed compared to under constant temperature (Roro *et al.*, Paper I). In response to 10 days of 6 h UV-B daily at 0.25 W m^{-2} in the middle of the light period under constant 20°C, the WT showed 20% reduction in shoot elongation (Fig. 8). When such a UV-B treatment was

provided together with the 6 h daily temperature drop, 46% reduction in shoot elongation was observed, compared to the control, and 30% reduction compared to temperature drop only (Fig. 8). Only temperature drop resulted in 23% reduction in shoot elongation. Thus, since the UV-B levels were about 25% lower during the temperature drop period, also this experiment might suggest that the UV-B response with respect to shoot elongation, at least to a certain extent, depends on the temperature regime.

The *lip1* mutant showed a similar response to the WT with 24% and 30% decrease, respectively, in shoot elongation after 10 days of 6 h daily UV-B exposure at 0.25 W m^{-2} or a 6 h daily temperature drop (conditions like for the WT) (Fig. 8). The combined treatment of UV-B and temperature drop reduced shoot elongation in the *lip1* mutant by 51% and 29% compared to the control not exposed to UV-B and the temperature drop only (Fig. 8). Accordingly, although the *lip1* mutant is dwarfed, this mutation did not alter the shoot elongation response to UV-B compared to the WT. This suggests that UV-B acts independently of LIP1 in modulation of shoot elongation.

The *le* mutant is GA-deficit due to mutation in the *GA3ox1*, which encodes the enzyme responsible for conversion of GA_{20} to the bioactive GA_1 (Ross *et al.*, 1989). This mutant is not completely devoid of GA, but contains very low levels. Shoot elongation in the *le* mutant was not significantly affected by the 6 h UV-B treatment at 0.25 W m^{-2} (Fig. 8). However, a slight effect of the 6 h temperature drop treatment with 11% decrease in shoot elongation in the *le* mutant was observed (Fig. 8). When UV-B was provided under the temperature drop, shoot elongation was reduced by 20% and 10%, respectively, compared to the control plants not exposed to UV-B and the temperature drop only (Fig. 8). Thus, although the *le* mutant was

unable to adjust its growth significantly in response to UV-B under constant temperature, the response to UV-B appeared to depend on temperature regime.

The *la cry-s* mutant, which is elongated and behaves like being GA saturated due to mutation in the two *DELLA* GA signaling genes in pea (Weston *et al.*, 2008), was not significantly affected by UV-B with respect to shoot elongation when exposed to 30 min or 6 h UV-B at 0.25 W m^{-2} under constant temperature or 6 h temperature drop treatment (Fig. 7-8). This, together with the lack of UV-B response in the *le* mutant (at least under constant temperature), demonstrates that ability to adjust the GA levels or GA response is required to respond to UV-B with decreased shoot elongation. The inability of the *la cry-s DELLA* mutant to regulate its elongation growth may be associated with that DELLA accumulation enhances LONG1 levels, as suggested with respect to HY5 in photomorphogenesis (visible light) in *A. thaliana* (Alabadi *et al.*, 2008). Consistent with the lack of reduced shoot elongation in response to UV-B in the *long1* mutant (Fig. 7), LONG1 accumulation is in turn required to enhance *GA2ox2* expression and thus decrease GA levels in response to UV-B (Weller *et al.*, 2009). Indeed, independently of exposure to light or darkness, the *long1* mutant was reported to contain high levels of bioactive GA_1 due to reduced *GA2ox2* activity (Weller *et al.*, 2009). Increased GA inactivation in pea in response to UV-B is consistent with our recent study where UV-B was shown to reduce the levels of bioactive GA_1, particularly through increased GA inactivation (Roro et al., Paper 1).

4.0. **Conclusions**

We have shown here that pea plants mutated in the pea homolog of the *A. thaliana* HY5, LONG1, behaves like the *hy5* mutant in being hypersensitive to UV-B-related damage and having low levels of specific flavonoids, and it does not show reduced shoot elongation in response to UV-B. Thus, like HY5 in *A. thaliana*, LONG1 in pea is as an important player in UV-B signaling resulting in formation of specific UV-B-protecting flavonoids and UV-B-induced inhibition of shoot elongation. The *A. thaliana* COP1 homolog in pea, LIP1, also appears to play a role in UV-B signaling with respect to production of flavonoids. However, opposite to *A. thaliana* plants mutated in *COP1*, the *lip1* mutant in pea exhibited enhanced UV-B-resistance and increased production of specific flavonoids compared to the WT. Furthermore, LIP1 does not appear to play a role in UV-B-induced reduction in elongation growth, since the *lip1* mutant, although dwarfed, showed a similar response in this respect as the WT. The dwarfed *le* GA biosynthesis mutant and the elongated *la cry-s* GA signaling mutant, which behaves like being GA saturated, were both more resistant to UV-B-related damage than the WT pea, probably due to higher levels of specific flavonoid glycosides. These observations, and that GA_3 application did not appear to affect the extent of UV-B-related damage, suggest that susceptibility to UV-B-related damage is not associated with GA levels or GA response and degree of elongation growth. However, ability to adjust the GA levels or GA response is apparently required for UV-B-induced reduction of elongation growth, as judged from the lack of UV-B induced reduction in shoot elongation in the *le* and *la cry-s* mutants. This study also supports that the response to UV-B with respect to shoot elongation in pea, at least to a certain degree, depends on temperature regime.

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Table 1. Phenolic compounds detected in pea leaves by HPLC analysis according to their appearance in the chromatograms.

Peak number	**Detected compound**	**Peak number**	**Detected compound**
1	Tryptophan	11	Kaempferol-glycoside
2	Unknown	12	Myricetin-glycoside
3	Quercetin-glycoside	13	Myricetin-glycoside
4	Kaempferol-glycoside	14	Apegenin-glycoside
5	Luteolin-glycoside	15	Unknown
6	Luteolin-glycoside	16	Phenolic acid
7	Luteolin-7- glycoside	17	Phenolic acid
8	Apigenin-7-glycoside	18	Phenolic acid
9	Luteolin-7-glycoside		
10	Apigenin-7-glycoside		

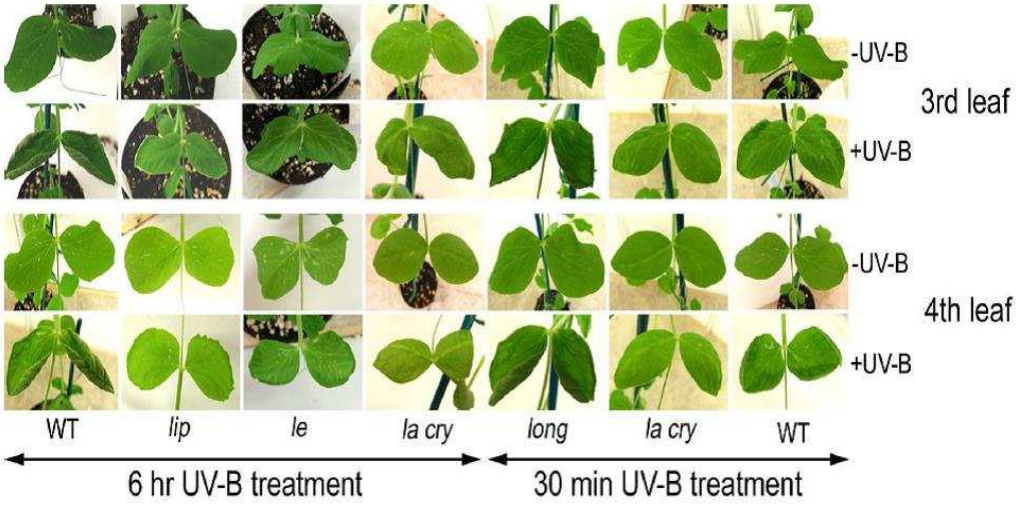

Fig. 1. Effect of a daily 30 min or 6 h UV-B treatment (+UV-B) at 0.25 W m^{-2} under a photosynthetic active radiation of 100 µmol m^{-2} s^{-1} in the middle of a 12 h light period for 10 days on the morphology of the 3rd and 4th leaf from the plant basis in the wild type (WT) ('Torsdag') and mutant plants of pea. The mutated genes are: *LONG1* and *LIP* which are homologs to *HY*5 and *COP1* in *Arabidopsis thaliana*, *LE* encoding the gibberellin (GA) biosynthesis gene *PaGA3ox1*, and *LA* and *CRY* encoding the two *DELLA* GA signaling genes described in pea. -UV-B denotes plants not exposed to UV-B.

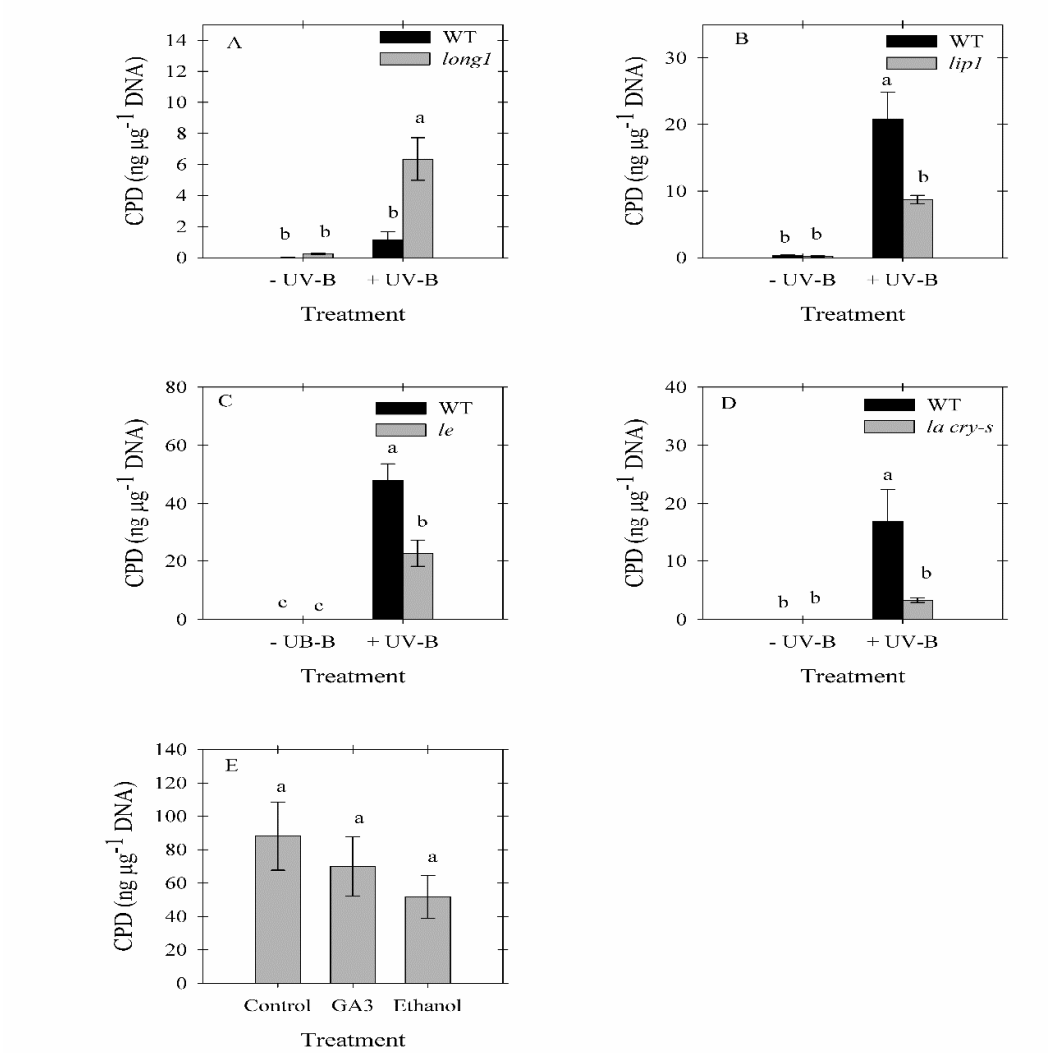

Fig 2. Effect of a daily 30 min (*long1* mutant compared to wild type (WT) (A)) or 6 h (*lip1*, *le* and *la cry-s* mutants, each compared to WT (B-D)) UV-B treatment (+UV-B) at 0.25 W m^{-2} (A-D) under a photosynthetic active radiation of 100 µmol m^{-2} s^{-1} in the middle of a 12 h light period for 10 days on levels of the DNA damage product cyclobutane pyrimidine dimers (CPD) in the 3rd leaf from the basis of the plant in WT and mutants of pea (A-D). CPD levels are also shown for WT pea applied with 10 µg GA_3 on the first unfolded leaf under 6 h daily UV-B exposure at 0.35 W m^{-2} (E) (Ethanol = mock treatment, control = UV-B without application; other conditions like for A-D). Values are mean ± SE of 5 plants in each of two repeated experiments. Different letters indicate statistically significant difference at $p \leq 0.05$.

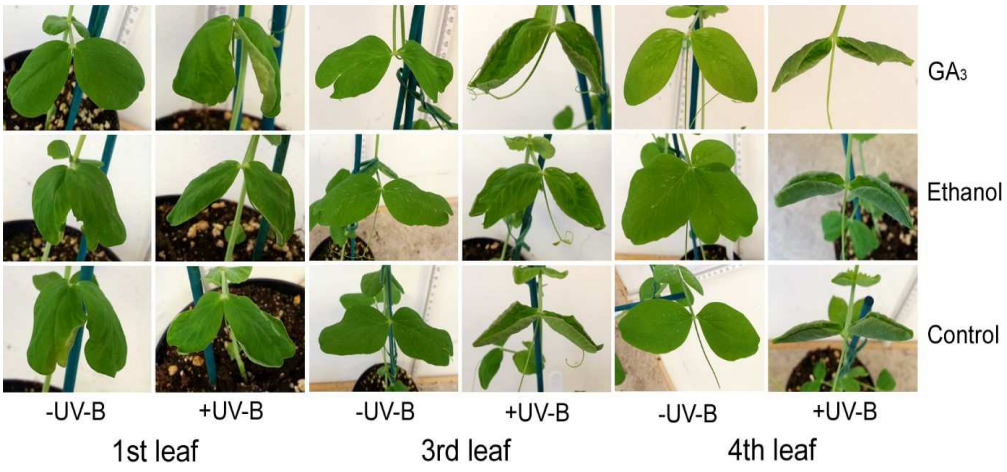

Fig. 3. Effect of application of 10 µg gibberellic acid (GA_3, dissolved in 10 ethanol) on morphology of the 1st (GA_3 applied), 3rd and 4th leaf in pea plants ('Torsdag') exposed to a daily 6 h UV-B treatment (+UV-B) at 0.35 W m^{-2} under a photosynthetic active radiation of 100 µmol m^{-2} s^{-1} in the middle of a 12 h light period for 10 days. Mock treated leaves with ethanol only and unapplied leaves (Control) are shown for comparison.

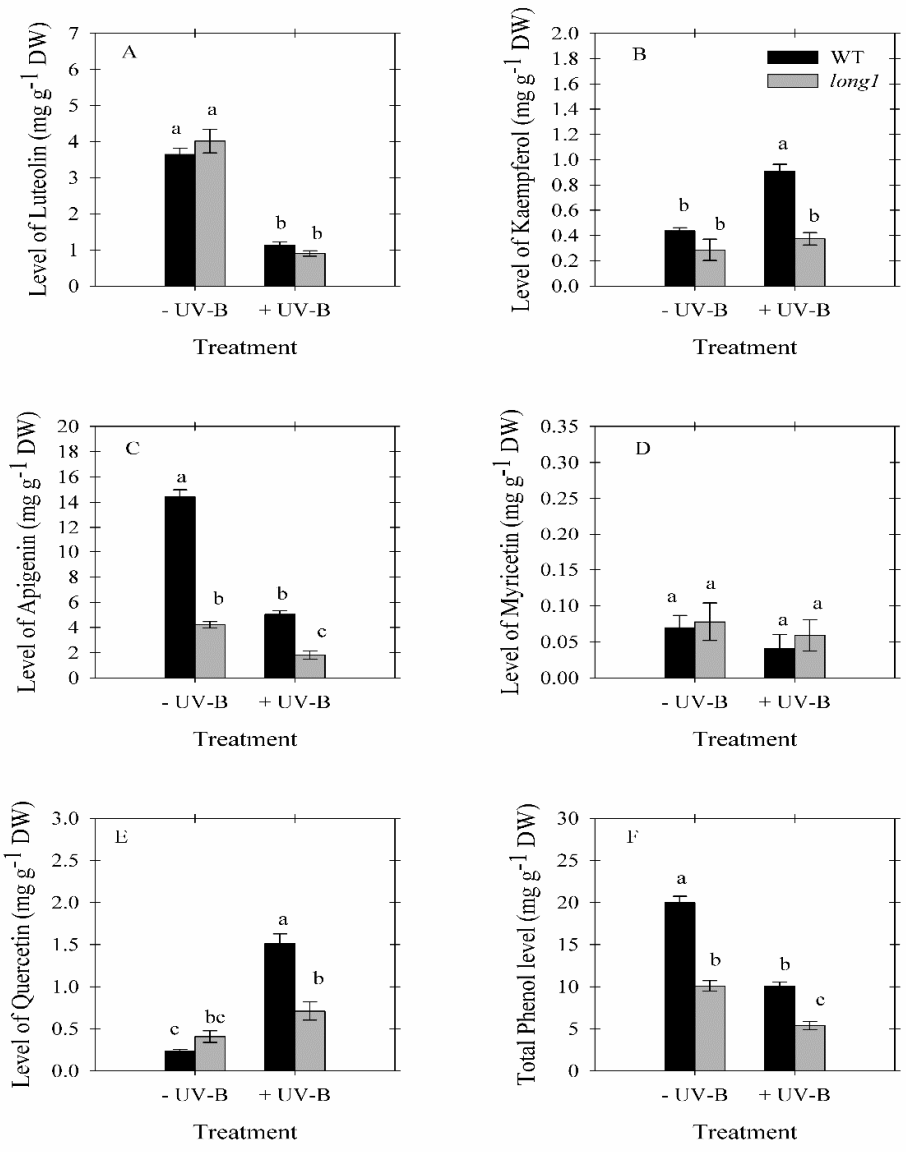

Fig 4. Effect of a daily 30 min UV-B treatment (+UV-B) at 0.35 W m^{-2} under a photosynthetic active radiation of 100 µmol m^{-2} s^{-1} in the middle of a 12 h light period for 10 days on levels of flavonoid glycosides (A-E) and total content of phenolic compounds (F) in the 3rd leaf from the basis of the plant in the *long1* mutant and wild type (WT) ('Torsdag') of pea. Values are mean ± SE of 10 plants in each of two repeated experiments. Different letters indicate statistically significant difference at $p \leq 0.05$.

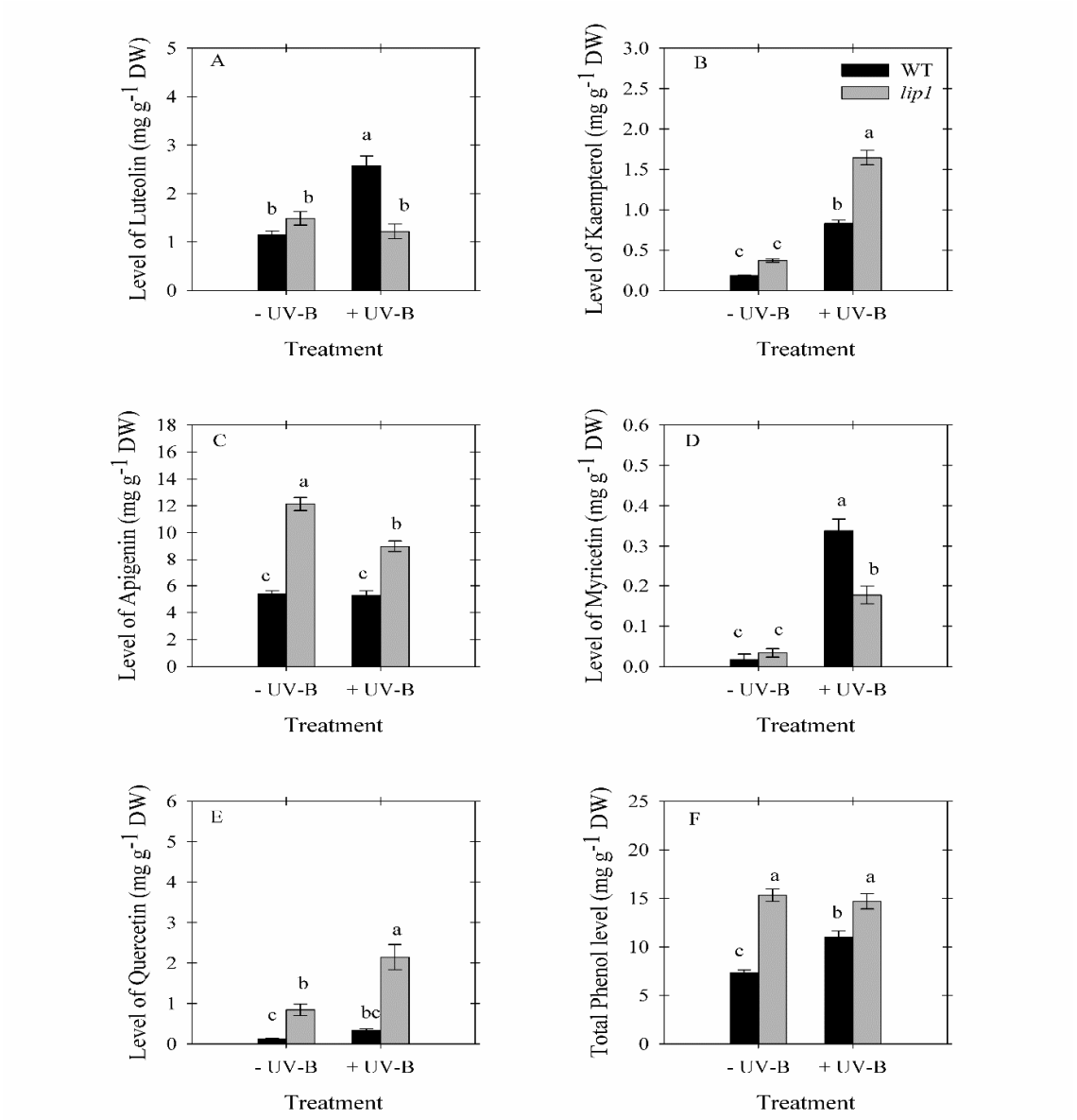

Fig 5. Effect of a daily 6 h UV-B treatment (+UV-B) at 0.5 W m^{-2} under a photosynthetic active radiation of 100 µmol m^{-2} s^{-1} in the middle of a 12 h light period for 10 days on levels of flavonoid glycosides (A-E) and total content of phenolic compounds (F) in the 3rd leaf from the basis of the plant in the *lip1* mutant and wild type (WT) ('Torsdag') of pea. Values are mean ± SE of 10 plants in each of two repeated experiments. Different letters indicate statistically significant difference at $p \leq 0.05$.

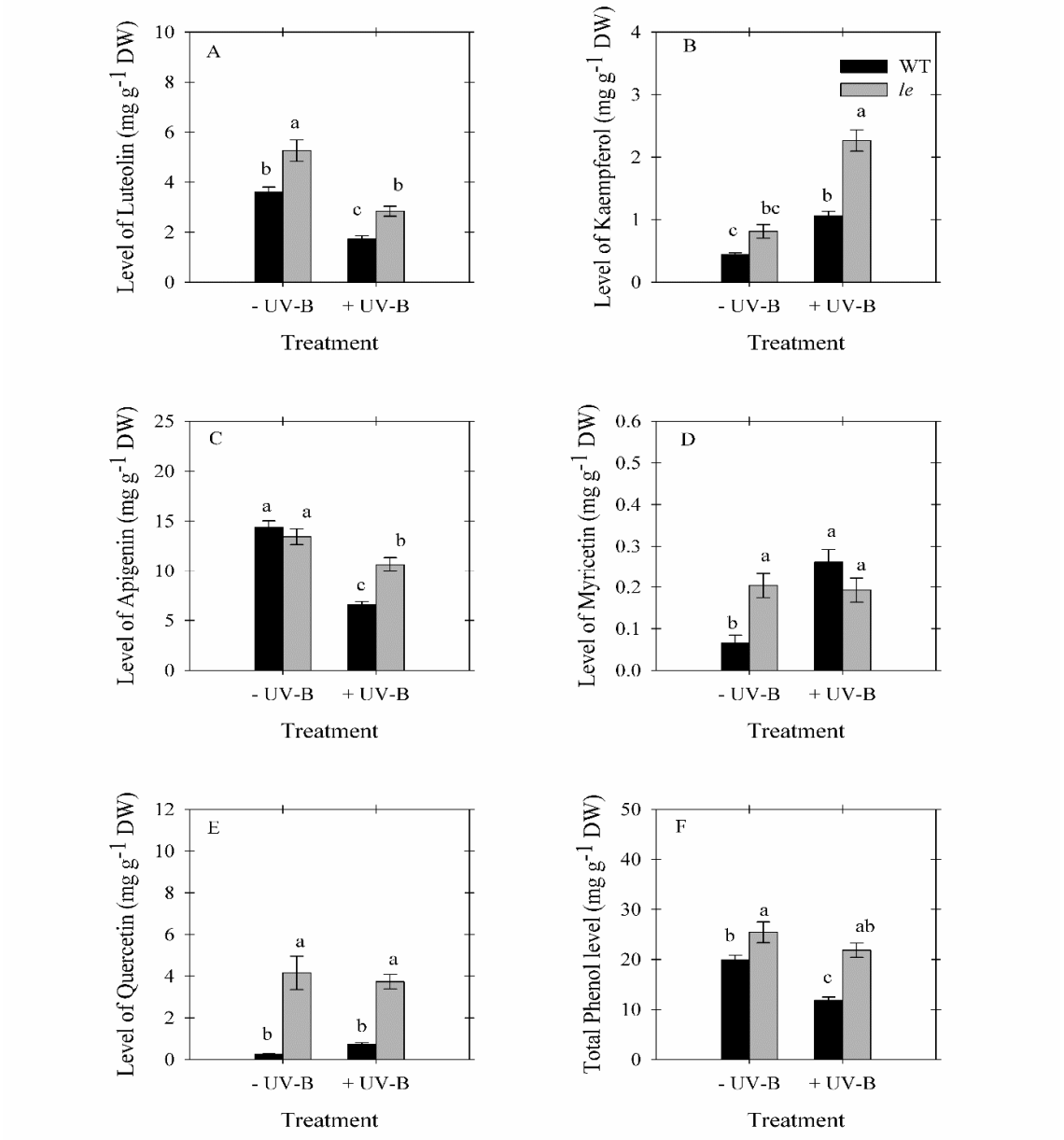

Fig 6. Effect of a daily 6 h UV-B treatment (+UV-B) at 0.35 W m^{-2} under a photosynthetic active radiation of 100 µmol m^{-2} s^{-1} in the middle of a 12 h light period for 10 days on levels of flavonoid glycosides (A-E) and total content of phenolic compounds (F) in the 3rd leaf from the basis of the plant in the *le* mutant and wild type (WT) (‘Torsdag’) of pea. Values are mean ± SE of 10 plants in each of two repeated experiments. Different letters indicate statistically significant difference at $p \leq 0.05$.

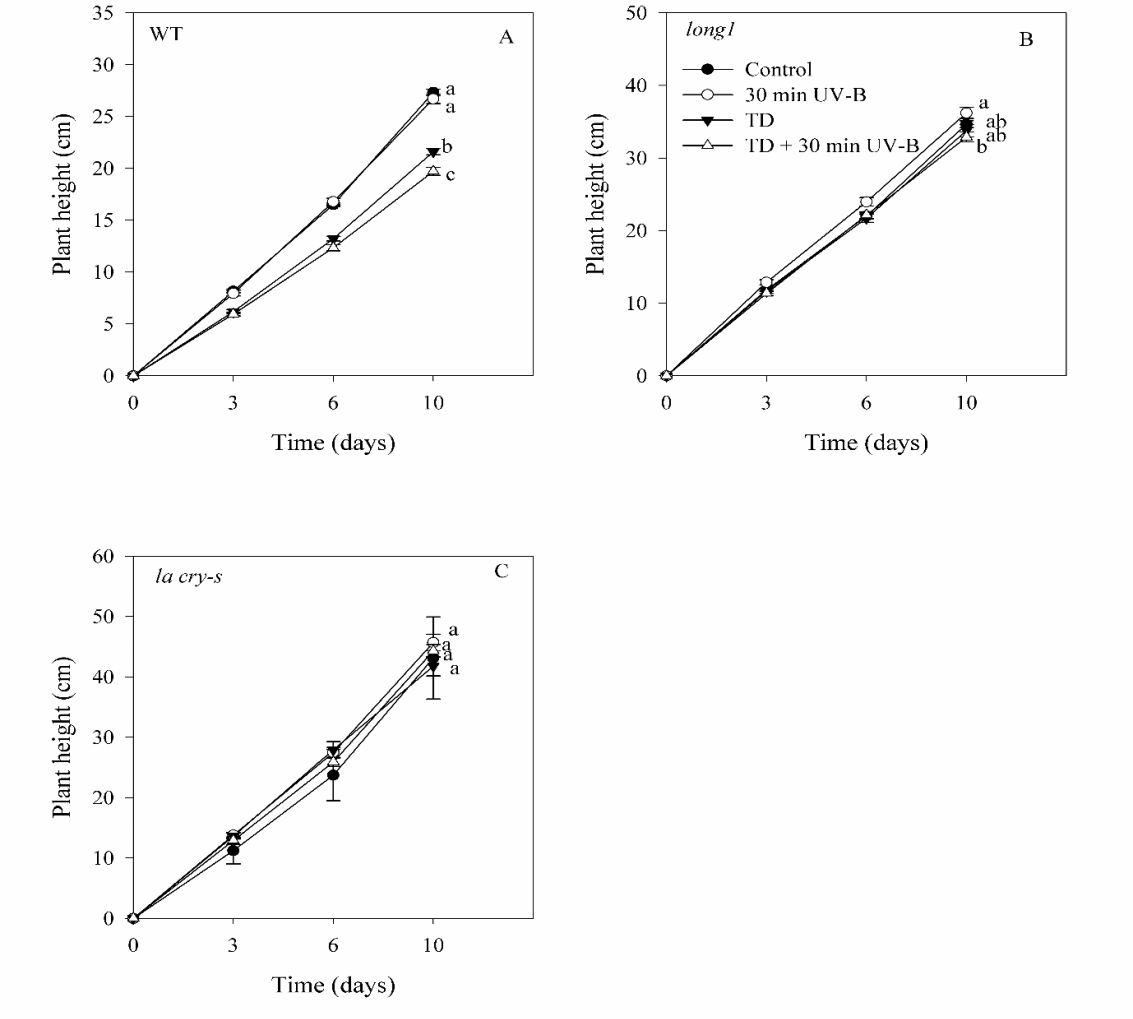

Fig 7. Effect of a daily 30 min UV-B treatment at 0.25 W m^{-2} under a photosynthetic active radiation of 100 µmol m^{-2} s^{-1} in the middle of a 12 h light period for 10 days on shoot elongation in the wild type (WT) ('Torsdag') (A), the *long1* mutant (B) and the *la cry*-s (C) mutant in pea. UV-B was provided under constant temperature of 20°C (Control = 20°C without UV-B) or in the middle of a 6 h temperature drop treatment (TD; 21°C to 13°C, otherwise 21°C; daily mean temperature = 20°C), given in the middle of the light period. Values are mean ± SE of 10-15 plants in each of two repeated experiments. Different letters indicate statistically significant difference at $p \leq 0.05$.

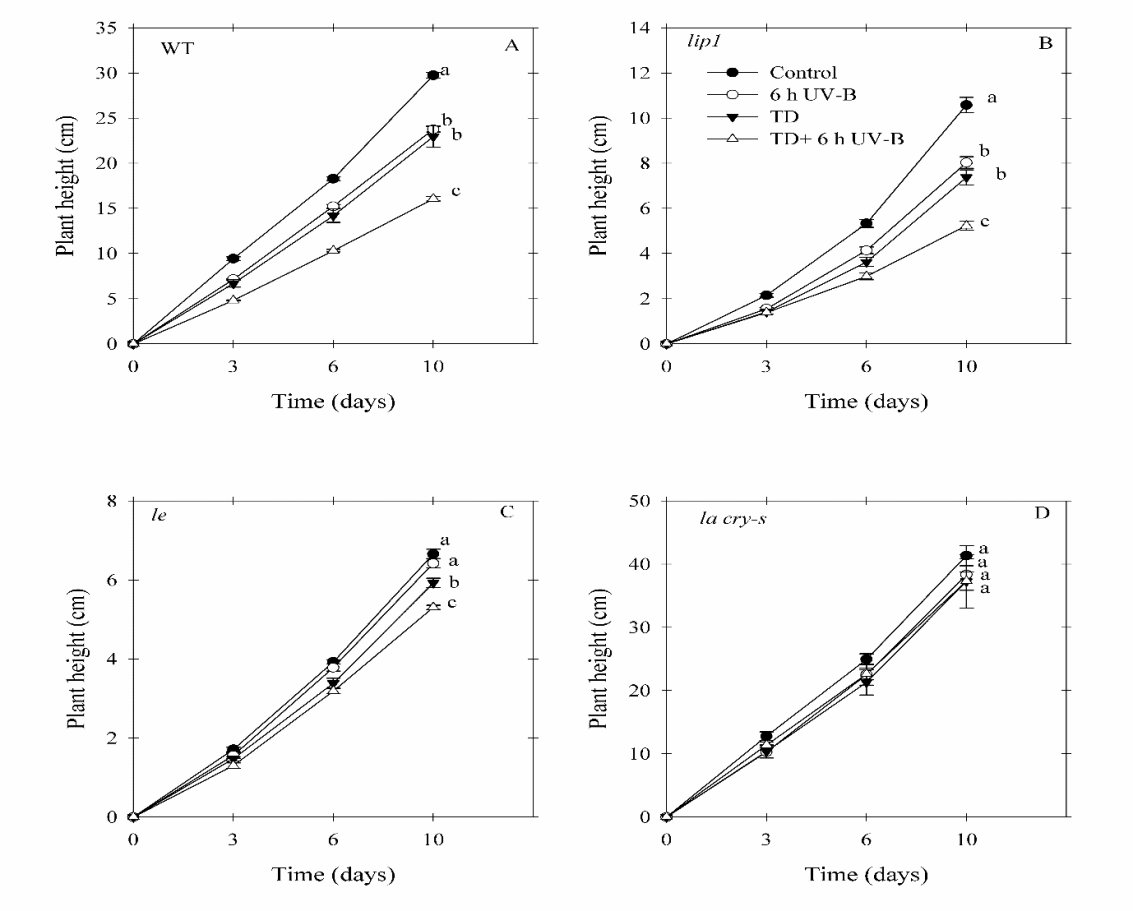

Fig. 8. Effect of a daily 6 h UV-B treatment at 0.25 W m^{-2} under a photosynthetic active radiation of 100 µmol m^{-2} s^{-1} in the middle of a 12 h light period for 10 days on shoot elongation in the wild type (WT) ('Torsdag') (A), the *lip1* mutant (B), le (C) and the *la cry*-s (D) mutant in pea. UV-B was provided under constant temperature of 20°C (Control =20°C without UV-B) or in the middle of a 6 h temperature drop treatment (TD; 21°C to 13°C, otherwise 21°C; daily mean temperature = 20°C) given in the middle of the light period. Values are mean ± SE of 10-15 plants in each of two repeated experiments. Different letters indicate statistically significant difference at $p \leq 0.05$.

Paper - III

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The impact of UV radiation at high altitudes close to the equator on morphology and productivity of pea (*Pisum sativum*) in different seasons

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ABSTRACT

Ultraviolet radiation (UV) is well known to affect plant growth and development and to vary with latitude and altitude. The knowledge about the effect of high UV levels at high altitudes close to the equator on plant productivity is scarce. By using UV-transmitting and UV-blocking films, the impact of solar UV on growth and production potential of commercial pea (*Pisum sativum*) was studied at a high (2800 meter above sea level (masl)) and a lower (1700 masl) altitude in Ethiopia during the dry (January-March) and wet (April-June) season. Morphological characteristics like plant height and number of branches as well as flowering time were affected by UV. Compared to the UV-blocking film, under the UV-transmitting film plants were 15-19% shorter and produced more branches at both altitudes and seasons. The flowering was delayed 2-5 days when exposed to UV but only minor differences was found in numbers of pods. Numbers of leaves and specific leaf area were important for pod number. These parameters were more affected by altitude and season than UV level. Also, stomatal

conductance at lower altitude was affected by season and was very low (0.06-0.08 mmol m^{-2} s^{-1}) during the dry season compared to wet season, irrespective of UV radiation. At higher altitude (2800 masl) UV radiation increased stomata conductance. Thus, the effect of UV on conductance depends largely on the interaction with other environmental conditions. Maximal PSII efficiency (Fv/Fm) was lowest in the dry season at both altitudes and the lowest value (0.66) was measured on plants exposed to UV radiation at high altitude. In conclusion, UV radiation affects plant morphology, flowering time, Fv/Fm and leaf conductance, but other climate factors, like irradiance, temperature and vapour pressure deficit (VPD), seems to have a stronger impact on productivity of pea than UV radiation.

Keywords:

Altitude

Morphology

Pisum sativum

UV-blocking film

UV-transmitting film

Season

Abbreviations: UV, Ultraviolet radiation; Fv/Fm, Maximal photosystem II efficiency; gs, Stomata conductance; PAR, Photosynthetically active radiation; RH, Relative air humidity; masl, meter above sea level; OPF, Open Field; SLA, specific leaf area; DAP = Diamonium phosphate; WUE, Water use efficiency

1. Introduction

The solar ultraviolet radiation (UV) at the earth's surface is an important environmental factor influencing growth and development of plants (Hollosy, 2002; Jansen, 2002; Jenkins, 2009). UV is traditionally divided into three wavelength ranges: UV-C (200-280 nm), which is extremely harmful to living organisms but not present in natural solar radiation at ground level and UV-B (280-315 nm) as well as UV-A (315-400 nm), which represent less than 1% and about 5% of the total incoming solar radiation, respectively, depending on cloud cover and atmospheric conditions (Hollosy, 2002). The distribution of UV on the ground surface is mainly affected by solar elevation, atmospheric air composition and cloudiness of the sky as well as altitude and latitude (Blumthaler *et al.*, 1994; Caldwell and Flint, 1994; Piazena, 1996; Foyo-Moreno *et al.*, 2003). Thus, even at a specific geographic location and season the amount of UV reaching the ground varies with the time of the day and day of the year.

Although exposure to high levels of UV-B may result in molecular and cellular damage due to the relatively high energy levels of these wavelengths (Jordan, 1996; Frohnmeyer and Staiger, 2003) a range of studies have demonstrated that rather than being a damaging stressor for plants, the UV-B reaching the earth`s surface exerts a range of regulatory effects (Hideg *et al.*, 2013). Long-term exposure of plants to UV-B may result in reduced leaf area, internode length and plant height (Barnes *et al.*, 1990; Antonelli *et al.*, 1997; Krizek *et al.*, 2006). Such morphogenetic effects can thus modify the water use efficiency (WUE) and structure of the vegetation. Water use efficiency of plant can also be related with the rate of gas exchange, which can be indirectly regulated by the aperture of the stomata pore, the number of stomata per leaves and speed of stomata movement (Hetherington and Woodward, 2003). A range of studies of different plant species have indicated that UV-B radiation affects stomatal movements and rate of opening (Day and Vogelmann, 1995; Tossi *et al.*, 2014). Furthermore, UV-B has been shown to affect the production of secondary metabolites, which due to their

protective functions against UV and a range of stressors, are of important physiological and ecological significance (Rozema *et al.*, 1997). Epidermal screening of UV-B through accumulation of secondary metabolites or reduction in leaf area can be a strategy for the plant to adapt to and escape from potentially harmful radiation (Blumthaler *et al.*, 1992; Jansen *et al.*, 1998). However, cultural plants may be more susceptible to UV-related damage because the level of UV-protecting compounds has been reduced by intensive breeding.

There are many reports showing an ameliorating effect of background light (photosynthetic active radiation (PAR), UV-A and blue light from UV-B related damage e.g. in species like soybean (*Glycine max*) and pea (*Pisum sativum*) (Strid *et al.*, 1990; Caldwell and Flint, 1994; Rozema *et al.*, 1997). Although the combined effects of temperature and UV-B on plants are not well documented, it has been reported that an increase in temperature (from 28 to 32°C) can increase the negative effect of UV-B on some growth parameters of crops (Teramura and Sullivan, 1994; Mark and Tevini, 1997). Furthermore, suspension-cultured tobacco cells (*Nicotiana tabacum*) has less UV-B related DNA-damage at lower than at higher temperature (Li *et al.*, 2002).

Ethiopia is located near the equator and about 50% of the total land is characterized as a mountainous region with elevations higher than 1500 m above sea level (masl) (Zeleke, 2010). Thus, since UV levels depend among others on the sun light`s distance to travel through the atmosphere and thus altitude, in such areas high levels of UV-B prevail at ground level compared to most other parts of the world where plants are grown (Sullivan *et al.*, 1992). Pea, which belongs to the *Leguminosae* family, is the second most important pulse crop in Ethiopia next to faba bean (*Vicia faba*) in terms of area and total production. It grows in most parts of the country, i.e. in middle (1800 masl) and high altitude (3000 masl) areas (between 3° -15°N and 33° - 48°E). Due to variation in agro-ecological conditions productivity of most crop species varies from region to region. In Ethiopia the wide range of variation in productivity

can be related to differences in climatic factors at different altitudes, i.e. such as different aspects of the light climate including UV, relative humidity (RH), precipitation and temperature (Bezabih and Sarr, 2012).

Although there are indications that pea plants irradiated with UV for a few hours may show reduced plant height, fresh and dry weight (Nogués *et al.*, 1998; Alexieva *et al.*, 2001), there is limited information on how pea productivity is affected by the high UV levels at high altitudes in areas close to the equator. Furthermore, although plants grown at high altitudes commonly show a more compact growth form than at lower altitudes (Went, 1953; Rawson, 1992; Körner, 2007) there is little information about interactive effects on plant morphology and plant productivity of UV at high altitudes and other environmental factors varying with altitude.

Using an approach with UV-transmitting and UV-blocking films, the aim of this study was to evaluate the effect of UV in different seasons (dry and wet) on vegetative growth, flowering and productivity of pea plants grown at two different high altitudes (1700 and 2800 masl) in Ethiopia. Under the UV-blocking film, UV-B and the shortest wavelengths of UV-A (lower than 350 nm) were almost absent. The climate at these altitudes differs in temperature, RH and solar radiation. Thus, this allowed us to evaluate the interactive effect of high UV-radiation and other climatic parameters differing with altitude and season.

2. Materials and methods

2.1. Plant materials and pre-growth

Seeds of pea (*Pisum sativum* L. *cv.* Cascade) was obtained from a commercial farm (Hadia flower and vegetable farm Addis Ababa, Ethiopia) and sown in pots (15 cm size) filled with coconut peat (Galuku Lankaexport PVt. Ltd, Kurunegala, Siri-lanka) and fertilized with 28 ppm Diamonium phosphate (DAP; $(NH_4)_2HPO_4$; 18% N, 46% P_2O_5), following the methodology of (Valenzuela, 1983). The pots were arranged under a shade house (25% shade) and subjected to similar environmental conditions, a temperature of 20°C ± 3°C and 70% RH, with 12/12 hour light/dark during germination of the seeds. Six days after germination pots containing plants of uniform size (1-2 cm shoot length) were transferred to the experimental sites.

2.2. Experimental locations and set-up

The field experiments were conducted at Hawassa (7º3'N 38º28E) at an altitude of 1700 meter masl and Hagereselam (6°27'N 38°27'E) at an altitude of 2800 masl. The experiments were conducted in the dry season (January-March 2012) as well as the wet season (April-June 2012). At each site the plants were grown either under UV-B-blocking film (Solar EVA- 5 High diffuse opaque film with 0.20 mm thick and 3 m wide Rovero plastic, Raamsdonksveer, The Netherlands) with selective cut-off of the solar spectrum below 350 nm (UV-B and the shortest wavelengths of UV-A) or UV-transmitting, polyethylene film (0.2 mm polyethylene sheet, Ethioplastic Pvt L.C, Addis Ababa, Ethiopia), which transmits wavelengths above 250 nm. Transmittance spectra of the two plastic films were measured at Norwegian University of

life sciences (NMBU) by illuminating the sample at the port of an integrating sphere (ISP-50-REFL Ocean Optics, Ocean Optics, Dunedin, Fla., USA) with a 600 μm thick optical fiber and a DH2000 (Ocean Optics) halogen light source. The light transmitted into the sphere was measured with a 400 μm fiber connected to an OceanOptics SD2000 spectrometer (Fig. 1). The plants were placed under the different filters covering small, 2 m high constructions, each of a total area of 9 m^2 (3 x 3 m). The bottom and top sides of the entire enclosure (15 cm above ground and 15 cm below roof) were left uncovered to allow ventilation. The structures were erected in North–South direction over the treatment plots. This orientation ensured that the solar radiation reached the plants only after passing through the filter as the sun moved from East to West.

2.3. Climate and radiation at the field sites

Weather data such as temperature, RH and sun shine duration of the last 10 years (2002 to 2011) were collected from the nearest meteorology station (Ethiopian national metrology agency, Hawassa and Hagereselam Branch). However, sun shine duration was only available from Hawassa (Table 6). During the study period, temperature and RH at the experimental sites (Table1) were recorded by mini data loggers (Testo 174, Version 5.0.2564.18771, Lenzkirch, Germany) every second week alternating between the two sites, starting in Hawassa. Each data logger was placed inside an open bucket to avoid direct sun and hanged close to the plant canopy (1 m above the ground). For statistical analysis the mean values of temperature and RH sampled during the four alternating weeks of measurement were considered for each site. UV-B (W m^{-2}) and PAR (μmol m^{-2} s^{-1}) were measured every hour from 6.00–18.00 on four randomly selected clear sky days using Skye spectrosense 2 with the sensors SKU 415 (PAR)

and SKU 430 (UV-B) Skye instruments, Llandrindod Wells, UK.). For statistical analysis, the mean values of PAR and UV-B obtained between 10:00 and 15:00 h were used.

Plants grown under UV-blocking plastic film will hereafter be referred to as minus UV (-UV), those grown under UV-transmitting plastic film referred to as plus UV (+UV) and those grown under unfiltered condition are denoted open field (OPF).

2.4.Plant growth analysis

During the experimental periods (79 days for each experiment) plant height, number of leaves and appearance of flower buds were recorded for six plants every 7 days. In each treatment, the visible flower buds were counted every week. At the end of the experiments (day 79) the total number of branches (>1 cm) and the numbers of pods per plant were counted. Only pods of a size equal to or longer than 4.5 cm were counted, since this corresponds to the commercial size of pods (Amurrio *et al.,* 1996). From the remaining six plants, leaves were detached and the total leaf area was measured with a LI-3100 leaf area meter (LI-COR, Inc., Lincoln, Nebraska, USA). Dry weight was determined after drying the leaves at 70℃ for 5 days and specific leaf area was calculated (SLA= leaf area/dry mass (cm^2gm^{-1}).

2.5. Chlorophyll fluorescence measurement

To evaluate the performance of the plants, maximal photosystem II efficiency (Fv/Fm) of well-developed leaves at the 4^{th}, 5^{th} and 6th node from randomly selected vegetative plants

(before flower buds appeared) was measured in the middle of the day with a Handy-*PEA* fluorimeter (Hansatech, Kings Lynn, UK) following the methodology of (Strasser *et al.*, 2004). Before measurement, leaves were dark-adapted in the leaf clip for 15 min. Light was then provided by an array of three high-intensity light-emitting diodes and adjusted to 1500 µmol m^{-2} s^{-1} to ensure that the photosynthesis was saturated during the measurements.

2.6. Stomata conductance

Stomata conductance (gs) was measured during the vegetative stage between the 5th and 6th week of the experiment on fully opened intact leaves at the 5th node using an open system LCA-4 ADC portable infrared gas analyzer (Analytical Development Company, Hoddeson, England). These measurements were done between 12:00 and 15:00 h with the following specifications/adjustments: Leaf surface area was 6.25 cm^2, ambient carbon dioxide concentration 340 µmol mol^{-1}, temperature of the leaf chamber varied from 34 to 47°C, leaf chamber molar gas flow rate was 410 µmol s^{-1}, ambient pressure 828 mbar and photosynthetic active radiation (PAR) at the leaf surface was maximum up to 1500 µmol m^{-2} s^{-1}. Data was collected every five min for 15 min using three leaves in each of 3 plants per treatment per experiment.

2.7. Statistical analysis

For each treatment in each experiment and experimental site, six plants from each plot were used for analysis (according to the description of data collection above). All statistical tests were performed in Minitab 16.1.1 (Minitab 16.1.1, windows version, State College, Pennsylvania, USA). Analysis of variance (ANOVA) was done using a completely randomized design and significant differences between means were tested using normally distributed general linear model (GLM) and Tukey's test. Correlation between pod, leaf number, total leaf area, specific leaf area (SLA), branch number and plant height were evaluated using stepwise regression analysis. Differences with $p \leq 0.05$ were considered significantly different.

3. Results

3.1. Climate data

The last 10 years (2002-2011) temperatures at the highest altitude (2800 masl), were lower with in average between 5.5℃ to 20℃ throughout the year, while at the lowest altitude (1700 masl) the temperature varied between 11℃-30℃ (Ethiopian National meteorological station, Hawassa and Hagereselam branch, Ethiopia). During the experimental period temperature showed similar trends and on average, the temperature at the lowest altitude was generally about 8°C higher than the temperature at the highest altitude. The average temperature in the dry season was 3-4°C higher than in the wet season at both altitudes. However, there was no statistically significant difference in temperature under the different films and there were no significant interactions between film, altitude and season. Compared to the lowest altitude, at the highest altitude a higher RH (lower vapour pressure deficit (VPD)) was measured under the filters both during the dry and wet season (Table1). However, filter type did not affect RH and there was no significant interaction between film and any of the other factors (altitude, season or film) (Table 1).

UV- transmitting and a UV-blocking filter removing UV-B and the shortest wavelengths of UV-A (Fig. 1) were used at the two altitudes (2800 and 1700 masl). Under each plastic film, PAR and UV-B at the experimental sites were measured during four randomly selected days during the dry and wet season. No significant difference in PAR levels at clear days were measured under the different films, but generally PAR was slightly higher at the highest altitude, especially during the dry season (Table 2). Slightly higher UV-B levels were measured under the UV-transmitting film at clear days at the highest altitude compared with the lowest altitude, especially during the dry season (Table 2). The levels of PAR under all films and UV-B under UV-transmitting films were reduced with approximately 50% compared with ambient irradiance levels (Fig. 2).

3.2. Plant growth and morphology

The elongation growth measured from the first week of treatment until week 5 is shown in Fig. 3 A and B. During the first three weeks small differences in growth between the treatments were found. However, from week 4 and 5 the differences between treatments become clearer (Fig. 3 A and B). At the highest altitude, under the UV-transmitting film, shoot elongation was reduced by about 19% and 15% during the dry and wet season, respectively, compared to under the UV-blocking film (Fig 3). Thus, the reduction was strongest during the dry season. At the lowest altitude shoot elongation was affected similarly by UV (no significant interaction between film and altitude), with 16% and 15% reduction in growth when UV was present in the dry and wet season, respectively (Fig 3). However, in this case there was no significant difference between the seasons. Removal of UV by UV-blocking filter had no effect on total leaf area and number of leaves per plant (Table 3). However, these parameters differed significantly between the seasons and there was a significant interaction between altitude and season for number of leaves regardless of UV-radiation (Table 3). At the highest and lowest altitude there were 4-5 and 8-9 more leaves per plant, respectively, in the wet compared to the dry season. Specific leaf area (SLA) was higher in the wet compared with the dry season except at the highest altitude under UV-transmitting film where the pattern was opposite (Table 3). Number of branches per plant was significantly affected by altitude, UV, season and an interaction between UV and season was found (Table 3). There was 33% and 40% more branches at the lowest compared to the highest altitude with and without UV radiation, respectively (Table 3). Similarly, during the dry season, plant grown under UV transmitting plastic film had more branches as compared to plant grown during the wet season.

3.3. Chlorophyll fluorescence and stomata conductance

Maximal photosystem II efficiency (Fv/Fm) was lower during the dry season than during the wet season, and the decrease in Fv/Fm was greater with UV than without UV at the highest altitude during the dry season, whereas there was no effect of season or UV on Fv/Fm at the lowest altitude. At the highest altitude stomata conductance was significantly higher when exposed to UV in both the dry and wet season. At the lowest altitude no such effect was observed but the conductance was much lower in the dry season compared to wet season, irrespective of UV (Table 4).

3.4. Flowering time and numbers of pods

There was a larger reduction in time to flowering in the wet compared with the dry season at the highest altitude than at the lowest altitude. At the lowest altitude, UV delayed time to visible flower buds with 4.5 and 4.8 days in the dry and wet season, respectively (Table 3). At the highest altitude plants showed flower buds 8-14 days later, depending on film and season, compared to plant growing at the lowest altitude. The longest flowering time was found in plants grown at the highest altitude in the dry season with UV present (69 days) (Table 3). At the highest altitude, in the dry season plants grown under the UV-blocking film had 4.7 more pods than plants grown under the UV-transmitting film. In the wet season at this altitude the number of pods was more similar under the different films (0.5 more with UV present). At the lowest altitude, compared to the UV-transmitting film there were 2.4 and 1.3 more pods under the UV-blocking film during the wet and dry season, respectively (Table 3). A correlation analysis was made with all growth parameters to evaluate the relation between growth parameters and yield of pea. The results showed that pod number was best explained by leaf number, SLA and plant height (R^2= - 0.96; $p \leq 0.002$, Table 5).

4. Discussion

4.1. Plant growth and morphology

In areas close to equator such as Ethiopia, commercial plant production is possible at high altitudes, but studies on the impact of UV radiation on plant growth and productivity at such conditions are scarce. In this study we investigated the effect of UV radiation at different altitudes on growth, development and productivity of pea plants. The films used in this experiment have different transmittance in the UV-B and UV-A spectral region. The UV-B blocking film cuts off all the UV-B spectral region (315-320 nm) and UV-A with shorter wavelengths than 350 nm, whereas the UV-transmitting film transmits all solar radiation (Fig. 1). However, also in the transmitting wavelength regions of the films the radiation is reduced by approx. 50% in the field probably due to dust (Table 2), whereas reduction through clean films in the lab is only approx. 20% (Fig. 1).

UV-B radiation is one of the solar spectrum components regulating plant responses including plant morphology (Jansen *et al.*, 1998; Jenkins, 2009). The results of this study with high natural UV levels at high altitudes close to the equator confirmed that plant morphological characteristics like plant height and number of branches were affected by UV radiation. Exclusion of UV-B and some UV-A from the solar spectrum enhanced the shoot elongation of pea plants by about 15-19% compared to unfiltered solar spectrum (Fig. 3). This is similar to a wide range of species where growth has been shown to be inhibited by solar UV-B (Caldwell and Flint, 1994; Krizek *et al.*, 1994; Teramura and Sullivan, 1994; Krizek *et al.*, 1998). Also, previous reports have demonstrated that supplementary UV-B radiation for extended periods of time either in controlled environment or field conditions result in significantly reduced shoot length in different plant species including crops like cucumber (*Cucumis sativus*), mung bean

(*Vigna radiata*), pot rose (*Rosa* x *hybrida*) and spinach (*Spinacia oleracea*) (Krizek *et al.*, 1994; Amudha *et al.*, 2005; Kumari *et al.*, 2009; Jayalakshmi *et al.*, 2011; Zlatev *et al.*, 2012; Terfa *et al.*, 2014). The reduction in shoot length and leaf area might be due an effect of UV-B on slowing down the rate of cell division (Hopkins *et al.*, 2002) and could be an adaptive mechanism to minimize the exposure area to UV radiation (Zlatev *et al.*, 2012). On the other hand, there are also reports on growth stimulation by UV (e.g. in tomato (*Solanum lycopersicum*) or no effect, e.g. in cotton (*Gossypium*) and oat (*Avena sativa*) (Caldwell and Flint, 1994; Krizek *et al.*, 1994; Teramura and Sullivan, 1994; Krizek *et al.*, 1998). Although the observed differences in responses between species might be due to species-specific characteristics, they may also well be due to aspects of the experimental conditions which can make comparison of results from different experiments difficult (Aphalo, 2012).

The plant growth of the different treatments before 4 weeks followed a similar growth pattern and the differences in plant height between the treatments discussed above were apparent after 4-5 weeks (Fig. 3 A and B). Then, the shortest plants were found at the highest altitude + UV irrespective of season (Fig. 3 A). The levels of PAR and UV-B were slightly higher at the highest altitude compared with the lowest altitude (Table 2). It has been reported that for every 1000 m increase in elevation, the global UV-irradiance (in the wavelength range between 300-320 nm) increases by 11% (Blumthaler *et al.*, 1997). In our investigation we measured an increase of 0.21 W m^{-2} from 1700 to 2800 masl during the dry season (Table 2). Thus, plants grown at the highest elevation were exposed to a higher irradiance and a higher intensity of UV radiation than plants grown at the lowest elevation. At the different altitudes the difference in temperature between dry and wet season was 2-4°C (Table 1) but plant height were reduced by 10-15% in both seasons at both altitudes, indicating that UV suppresses plant height irrespective of the background temperature. Also in a similar study of roses (Terfa *et*

al., 2014) shoot length was reduced when exposed to natural UV radiation at the same field sites as those of the present study, i.e. a higher and lower altitude

Compared to the unexposed control plants, more branches were found in plants exposed to UV (Table 3). Reduced apical dominance and stimulated branching is a characteristic growth pattern found in plants exposed to UV (Jansen, 2002). However, unlike a range of earlier reports (Barnes *et al.*, 1990; Barnes *et al.*, 1996; Krizek *et al.*, 1997), there was no significant effect of UV on total leaf area or number of leaves (Table 3). However, these parameters were significantly affected by season and an interactive effect between altitude and season was found for the number of leaves. The main difference in climate between altitudes and seasons, except for light climate, are temperature and VPD (Table 1). As expected, the temperature decreased with increasing altitude (Lippok *et al.*, 2013) with on average a 0.7°C increase every 100 m from 1700 to 2800 masl, whereas VPD decreased with altitude (Table 1). The lower leaf area (12-64%) and the lower numbers of leaves (21-44%) correspond with higher temperatures and lower RH (higher VPD), especially in the dry compared to the wet season (Table 1 and 3). A low VPD commonly increases fresh weight and leaf area of different plant species (Mortensen, 2000). Thus, the strong decrease in number of leaves and total leaf area at the lowest altitude during the dry season are probably related to a very high VPD. At the lowest altitude in the dry season the VPD was 2.2-2.3 KPa (Table 1 and 4). In most plant species, increasing the VPD to such high values around the leaf results in stomata closure (Turner *et al.*, 1984). However, at the highest altitude the VPD was in general smaller (0.42-1.02 KPa) and no direct relationship between VPD and leaf number was found. Nevertheless, at the highest altitude stomatal conductance was higher in plants produced with UV radiation compared to plants without UV. The fact that UV radiation increased stomatal opening at the highest but not the lowest altitude indicates interplay with other climate factors. Previously, contradictory effects of UV radiation on stomatal movements has been reported. In several studies UV-B has been found to induce

stomata closure and thus reduce stomata conductance (Mirecki and Teramura, 1984). On the other hand, UV-B irradiation has also been found to increase gas exchange through enhancement of the stomata openings (Musil and Wand, 1993; Dai *et al.*, 1995; Zeuthen *et al.*, 1997; Keiller and Holmes, 2001; Julkunen-Tiitto *et al.*, 2005). The effect of UV-B on stomata behavior is dependent on fluence rate. In general, very low fluence rate stimulates stomatal opening whereas a higher dose induces closure (Nogués *et al.*, 1999; Jansen and Van Den Noort, 2000; Eisinger *et al.*, 2003; He *et al.*, 2005; He *et al.*, 2013). However, the different stomatal response to UV in the present study is rather an effect of the background climate than the UV-B dose. A wide range of studies have reported that the stomatal response to UV depends largely on different environmental factors, such as background light climate and soil water content (Nogués and Baker, 2000; Eisinger *et al.*, 2003).

SLA was affected by the combined effect of UV and season (Table 3). Variation in SLA might be due to variation in leaf thickness or leaf density (Veneklaas *et al.*, 2002). Under natural growing conditions with UV present, different reports have shown that SLA vary with leaf age (Reich *et al.*, 1992; Coleman *et al.*, 1994; Reich *et al.*, 1999), altitude and length of the growing season (Körner, 2007). However, leaf development is not necessarily linear, e.g. (Li *et al.*, 2006) reported that an increase in SLA in *Quercus aquifolioides* plants increased with increasing altitude until 2800 masl, but at the highest elevation (about 3600 masl) SLA was reduced by about 45% compared to at 2800 masl. Moreover, the report by (Moser *et al.*, 2007) indicated that the average SLA in forest stands recorded at different altitudes (1050, 1880, and 2380 masl) was significantly different at different altitudes, with up to 40 % higher SLA at the lowest (1050 masl) compared to highest (2380 masl) altitude. In our study also the significantly different irradiance levels (PAR) in the different seasons and altitudes (Table 2), had probably affected SLA. Higher PAR in the dry season generally (except for at +UV at the highest altitude) correlated with decreased SLA (Table 2 and 3). This corresponds with the

investigation of (Meziane and Shipley, 1999), in which a strong negative correlation was observed between SLA of different herbaceous plants and the level of irradiance.

Increasing stomata conductance, stomata frequency and leaf thickness are found in many plant species with increasing elevation (Körner *et al.*, 1986). Such changes in leaf characteristics with altitude might be due to fluctuation in temperature and amount of light intercepted by the leaf. On the other hand, Fv/Fm values of the plants measured in the dry season was lower at the highest altitude under the highest VPD. We found the lowest Fv/Fm (highest stress; Fv/Fm= 0.66) with solar UV present at the highest altitude during the dry season (Table 4). It is likely that the high UV and/or PAR levels at the highest altitude resulted in photoinhibition measured as reduced Fv/Fm (Table 4). Moreover, other reports indicated a negative correlation between irradiance and Fv/Fm ratio in plant species grown in field (Dawson and Dennison, 1996). Thus, not only UV-B but probably also the combined effect of high levels of PAR and high air temperature in the dry season reduced the Fv/Fm value of pea plant at high altitude. However, there was no clear relationship between number of pods and Fv/Fm.

4.2. Time to visible flower buds and number of pods

To evaluate the combined effect of altitude, film and season on plant productivity we also counted the number of days to appearance of the first flower bud and the total number of pods produced per plant at the end of the experiment (at day 79; Table 3). Although flowering time in most plant species vary with genetic as well as environmental factors, flowering in pea has been reported to commonly start about 40-50 days after planting in the field (McKay *et al.*, 2003). This was the case also in these experiments and the first plants flowered after 45 days (Table 3).Under the UV-transmitting film, time to visible flower buds was significantly delayed

by 2.5-4.8 days. The earliest flowering was found when UV-B and the shortest wavelengths regions of UV-A was excluded from the solar spectrum. Delay in time to flowering under high UV-B radiation has previously been reported in different plant species including crops like maize (*Zea mays*), petunia and roses (Staxén and Bornman, 1994; Saile-Mark *et al.*, 1996; Caldwell *et al.*, 1998; Terfa *et al.*, 2014). In the study of Terfa et al. (2014), with a similar set-up as the present study, flowering of roses was delayed 7-10 days with UV as compared to -UV radiation at both altitudes (same sites as the present study). Terfa et al. (2014) claimed that the delay in flowering might be an indirect effect of UV radiation, because of reduced leaf area resulting in lower light capturing and lower dry matter accumulation. In our study with pea no differences in total leaf area was found between +UV and -UV like in roses. Thus, the delayed flowering under +UV might be stress related. A strong negative correlation was found between flowering time and Fv/Fm value (Pearson correlation: -0.897, p=0.001) and indicates delayed flowering with decreased Fv/Fm value.

Variation in yield, flowering and pod production in pea due to seasons and temperature has been reported by different researchers (Ridge and Pye, 1985; French, 1990; McDonald and Paulsen, 1997). A study of the field-grown tropical legume *Cyamopsis teragonoloba var. Pusa navagar* showed that plants grown under ambient UV-B radiation had delayed onset of flowering and reduced pod size by 60% as compared to plants grown without UV-B radiation (Amudha *et al.*, 2005). Similarly, (Chimphango *et al.*, 2007) reported delayed flowering time and lower yield in UV-B-exposed soybean. Although flowering time was affected in our study, the number of pods was not much affected by UV-radiation. Rather, results from our study revealed that, the number of pods per plant at the end of the experiments was strongly affected by season, and only slightly affected by UV. The most important growth parameters to explain pod number per plant was leaf number, then SLA and plant height (Table 5). The pod number

per plant negatively correlated with number of leaves, size of SLA and plant height (R^2 = -0.96, $p \leq 0.002$, Table 5).

The fact that plants with more leaves and lower SLA had fewer pods points towards a competition or a changed balance between vegetative and generative growth like shown in other crops i.e. tomato and fruit trees (Heuvelink and Buiskool, 1995; Marcelis *et al.*, 1998). High SLA is a trait that is often associated with relatively high growth rate, development of young leaves and production of small seed mass (Grotkopp *et al.*, 2002). Leaves with a lower SLA are thicker and usually have a higher density of chlorophyll and protein per unit leaf area and hence, a greater photosynthesis capacity (Poorter and Evans, 1998; Evans and Poorter, 2001). In this study it was observed that pea leaves with lower SLA was more efficient in pod production than pea plant with higher SLA. Further, a smaller leaf size results in reduced boundary layer resistance that helps to maintain favorable leaf temperatures and improve efficient water usage under high solar radiation. Low SLA has been found to maintain a higher relative water content in leaves and it is assumed to be a way to improve WUE (Craufurd *et al.*, 1999; Nautiyal *et al.*, 2002). Water usage was not measured in this study but production of a higher biomass per unit of water transpired is an important physiological parameter in sustainable production of pea pods. Further work is required to study effects of UV radiation on WUE.

5. Conclusions

Using an approach with UV-transmitting and UV-blocking filters, this study shows that UV radiation caused changes in morphology and flowering time but had minor effects on pod number. Pea plants exposed to UV radiation had shorter stems with more branches and later flowering irrespective of altitude and season. A strong correlation was found between number of leaves and number of pods. The number of leaves was more affected by altitude and season than UV radiation. Thus, other climate factors (PAR, temperature and VPD) may have a stronger effect on productivity of pea than UV radiation.

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Fig 1. Solar spectrum transmission of polyethylene films used in the growth experiment with pea: UV-blocking polyethylene film (-UV) (solid line) blocks UV-B spectrum (280-315 nm) and the short wavelengths of UV-A (≤ 350) (Solar EVA-5 0.20 mm thick high diffuse opaque polyethylene film, Rovera plastic, The Netherlands). UV-transmitting (+UV) polyethylene film (dotted line) transmitting the solar spectrum beyond 250 nm (0.2 mm film, Ethioplastic Pvt LC., Addis Ababa, Ethiopia).The light transmitted through the plastic film was measured with a 400 µm fiber connected to an Ocean-optics SD2000 spectrometer.

Fig 2. UV-B distribution under UV-transmitting (+UV), UV-blocking (-UV) film and open field (OPF) during the dry season (A, B and C; January-April) and wet season (D; April-June) on four randomly selected days in 2012 at the field sites in Ethiopia at a higher (2800 masl) and lower (1700 masl) altitude. Each point represents the average value of six measurements taken on randomly selected days.

Fig 3. Plant height from 0 week to 5 week of growth (A and B) were measured in pea plant grown under UV-transmitting (+UV) and UV-blocking (-UV) plastic films at a higher (2800 masl) and lower (1700 masl) altitude in Ethiopia during dry (January –April) (A) and wet (April –June) (B) seasons. At each site and season, data are the mean values ± SE of measurements from six plants. All values sharing the same letter are statistically non-significant at $p \leq 0.05$.

Table 1. Climate data sampled under UV-transmitting (+UV) or UV-blocking (-UV) films during the dry (January-February 2012) and wet (April-June 2012) season at a higher (2800 masl) and lower (1700 masl) altitude in Ethiopia. The mean temperature (T_{mean} ℃) and the relative air humidity (RH) were logged every second week alternating between the two sites starting in Hawassa by a mini data logger (Testo 174) at the top of plant canopy. Finally, water vapor pressure deficit (VPD) was calculated based on the recorded temperature and relative air humidity.

Altitude	Plastic film	Season	T_{mean} (°C)	RH (%)	VPD (KPa)
	+UV	Dry	19.5±3.0bc*	56.5±2.2bc	1.02±0.24bc
		Wet	16.5±0.1c	77.7±0.5a	0.42±0.01c
High		Mean	18	67.1	0.72
	-UV	Dry	20.2±2.6abc	55.5±0.5c	1.1±0.18bc
		Wet	16.6±0.4c	76.7±0.6a	0.44±0.02c
		Mean	18.4	66.1	0.77
	+UV	Dry	27.5±0.0ab	40.6±2.6d	2.2±0.10a
		Wet	25.3±0.4ab	63.4±0.4bc	1.2±0.02b
Low		Mean	26.4	52	1.7
	-UV	Dry	27.8±0.0a	39.1±2.7d	2.3±0.10a

		Wet	24.0±0.4abc	65.8±2.5b	1.02±0.10bc
		Mean	25.9	52.5	1.66
p-Value	Altitude		0.001	0.001	0.001
	Film		0.973	0.845	1.00
	Season		0.014	0.001	0.001
	Altitude x Film		0.670	0.589	0.672
	Altitude x Season		0.871	0.205	0.017
	Film x season		0.602	0.467	0.436
	Altitude x Film x season		0.787	0.476	0.555

* All values sharing the same letter in a column are statistically non-significant at $p \leq 0.05$.

Table 2. Ambient irradiance levels and irradiance levels of UV-B (W m^{-2}) and photosynthetic active radiation (PAR) (µmol m^{-2} s^{-1}) below UV transmitting (+UV) and UV blocking films (-UV) measured in the middle of the day (11:00 to 15:00) at a lower (1700 masl) and higher (2800 masl) altitude in Ethiopia during dry (January –April) and wet (April - June) seasons. Percent reduction in irradiance below films compared with ambient irradiance levels is also shown. The average monthly sun shine duration for dry (January – March) and wet (April to June) season for the period 2002 – 2011 was calculated based on the secondary data obtained from the nearest meteorological station (Ethiopian national metrology agency, Hawassa Branch).

Altitude	Film	UV-B ambient (W m^{-2})	UV-B below film (W m^{-2})	% UV-B reduction	PAR ambient (µmol m^{-2} s^{-1})	PAR below film (µmol m^{-2} s^{-1})	% PAR reduction	Av. Sun shine duration (h)
				Dry season				
1700	+UV	2.20	0.82	63	1910	948	50	8.8
	-UV		0.05	98		1067	44	
2800	+UV	2.41	0.90	63	1919	1028	46	
	-UV		0.05	98		1105	42	
				Wet season				

1700	+UV	0.93	0.56	40	1051	600	43	6.9
	-UV		0.04	96		676	36	
2800	+UV	2.00	0.89	56	1668	960	42	
	-UV		0.04	98		825	51	

Table 3. Growth parameters, number of days to visible flower buds and pod number per plant of pea grown for 79 days under UV-transmitting (+UV) and UV-blocking (-UV) plastic films at a higher (2800 masl) and lower (1700 masl) altitude in Ethiopia during the dry (January-April) and wet (April-June) season in 2012. At each site and season, data are the mean values ±SE of measurements from six plants.

Altitude	Plastic film	Season	Total leaf area (cm^2)	Number of leaves	Branch number $plant^{-1}$	Specific leaf area ($cm^2\ g^{-1}$)	Days to flowering	Pods $plant^{-1}$
High	+UV	Dry	350±56.7ab*	13.5±0.67bc	2.0±0.3b	407.6±53.4a	69.3±1.48a	9.8±1.14bc
		Wet	411.3±10.5ab	18.3±1.9ab	2.1±0.2b	310.9±7.4ab	60.5±0.99bc	8.2±1.35c
		Mean	380.7	15.9	2.05	359.3	64.9	9.0
	-UV	Dry	429±106ab	13.3±0.5bc	1.2±0.2c	255.9±7.4b	64.7±0.67b	14.5±1.7ab
		Wet	488.2±60.5ab	17.20.95abc	2.1±0.1b	346.4±18.7ab	58.0±1.24cd	7.7±0.76c
		Mean	458.5	15.3	1.65	301.2	61.4	11.1
Low	+UV	Dry	304.2±11.4ab	11.7±0.76c	3.0±0.2a	266.9±16.3b	55.0±0.78de	14.0±1.2ab
		Wet	414±48.9ab	21.0±2.8a	3.0±0.2a	341.2±35.1ab	52.2±0.95ef	5.3±0.9c
		Mean	359.1	16.4	3.0	304.1	53.6	9.7

	-UV	Dry	210.2±73.9b	11.7±1.09c	2.0±0.2b	248.7±15.4b	50.5±0.50fg	15.3±1.3a
		Wet	577.7±108a	19.5±1.28ab	2.3±0.2ab	377.0±39.3ab	47.3±0.88g	7.7±0.84c
		Mean	394.0	15.6	2.15	312.9	48.9	8.0
p-Value	Altitude		0.390	0.714	0.001	0.316	0.001	0.523
	Film		0.263	0.490	0.001	0.257	0.001	0.025
	Season		0.007	0.001	0.015	0.027	0.001	0.001
	Altitude x Film		0.664	0.967	0.111	0.126	0.438	0.882
	Altitude x Season		0.086	0.043	0.167	0.019	0.001	0.025
	Film x season		0.207	0.542	0.026	0.008	0.512	0.222
	Altitude x Film x season		0.201	0.903	0.242	0.128	0.372	0.074

* All values sharing the same letter in a column are statistically non-significant at $p \leq 0.05$

Table 4. Stomata conductance and maximal photosystem II efficiency of fully developed pea leaves measured in the middle of the day at a higher (2800 masl) and a lower (1700 masl) altitude in Ethiopia during the dry and wet season (2012) under UV-blocking (-UV) or UV-transmitting (+UV) plastic film. Leaves on the 5th node of randomly selected plants were used for determination of stomata conductance. Photosystem II efficiency was measured during the vegetative growth stage on the 4^{th}, 5^{th} and 6^{th} node of randomly selected three plants under each film. The values are mean ±SE of three leaves in each of three plants (n=9).

Altitude	Plastic film	Season	Stomata conductance (mmol m^{-2} s^{-1})	Fv/Fm
High	+UV	Dry	0.31±0.04a*	0.66±0.03c
		Wet	0.28±0.02ab	0.78±0.005a
		Mean	0.30	0.72
	-UV	Dry	0.17±0.02c	0.72±0.009b
		Wet	0.15±0.01cd	0.78±0.007a
		Mean	0.16	0.75
Low	+UV	Dry	0.08±0.01de	0.77±0.008ab
		Wet	0.15±0.01cd	0.82±0.004a

		Mean	0.12	0.80
	-UV	Dry	0.06±0.01e	0.79±0.009a
		Wet	0.20±0.02bc	0.81±0.005a
		Mean	0.13	0.80
p- Value				
Altitude			0.001	0.001
Film			0.001	0.044
Season			0.002	0.001
Altitude x Film			0.001	0.233
Altitude x Season			0.001	0.002
Film x season			0.193	0.014
Altitude x Film x season			0.180	0.438

* All values sharing the same letter in a column are statistically non-significant at $p \leq 0.05$

Table 5. Growth parameter components contributing to variation in pod production (pods per plant) in pea (*Pisum sativum*) grown in Ethiopia at a higher (2800 masl) and lower (1700 (masl) altitude (stepwise multiple regression with α =0.05 was used as a criterion for acceptance, or rejection of model).

Parameters	steps	S	R^2	R^2 (adj)	Mallows Cp	p-value	Stepwise regression model
Leaf number	1	1.37	85.6	85.3	117.4	0.001	Pod= 29.41+0.083(PLH)-0.91(LNo)-0.0214(SLA)
Leaf number + SLA	2	0.805	95.12	94.9	12.7	0.001	
Leaf number + SLA + Plant height	3	0.730	96.07	95.8	4.0	0.002	

Where: LNo = Number of leaves; PLH= Plant height; SLA =Specific leaf area; S=standard error of estimate; R^2= R square

Table 6. Average values of minimum (Min) and maximum (Max) temperature (℃), RH (%), sunshine duration (h), wind speed (m s^{-1}) and rain fall (mm) for the past ten years (2002 to 2011) recorded by two different meteorological stations of southern Ethiopia located nearest to the study area at a higher (2800 masl) and lower (1700 masl) altitude (Ethiopian National meteorological station, Hawassa and Hagereselam branch).

Climate parameters	Lowest altitude		Highest altitude	
	Min	Max	Min	Max
Temperature (℃)	10.9	30.1	5.5	20.7
Rain fall (mm)	25.3	129.4	27.9	157.6
Relative humidity (RH %)	54.9	73.2	NA*	NA
Sunshine duration (h)	5.0	9.3	NA	NA
Wind speed (m s^{-1})	0.6	1.0	NA	NA

∗ NA= Data not available

Fig 1.

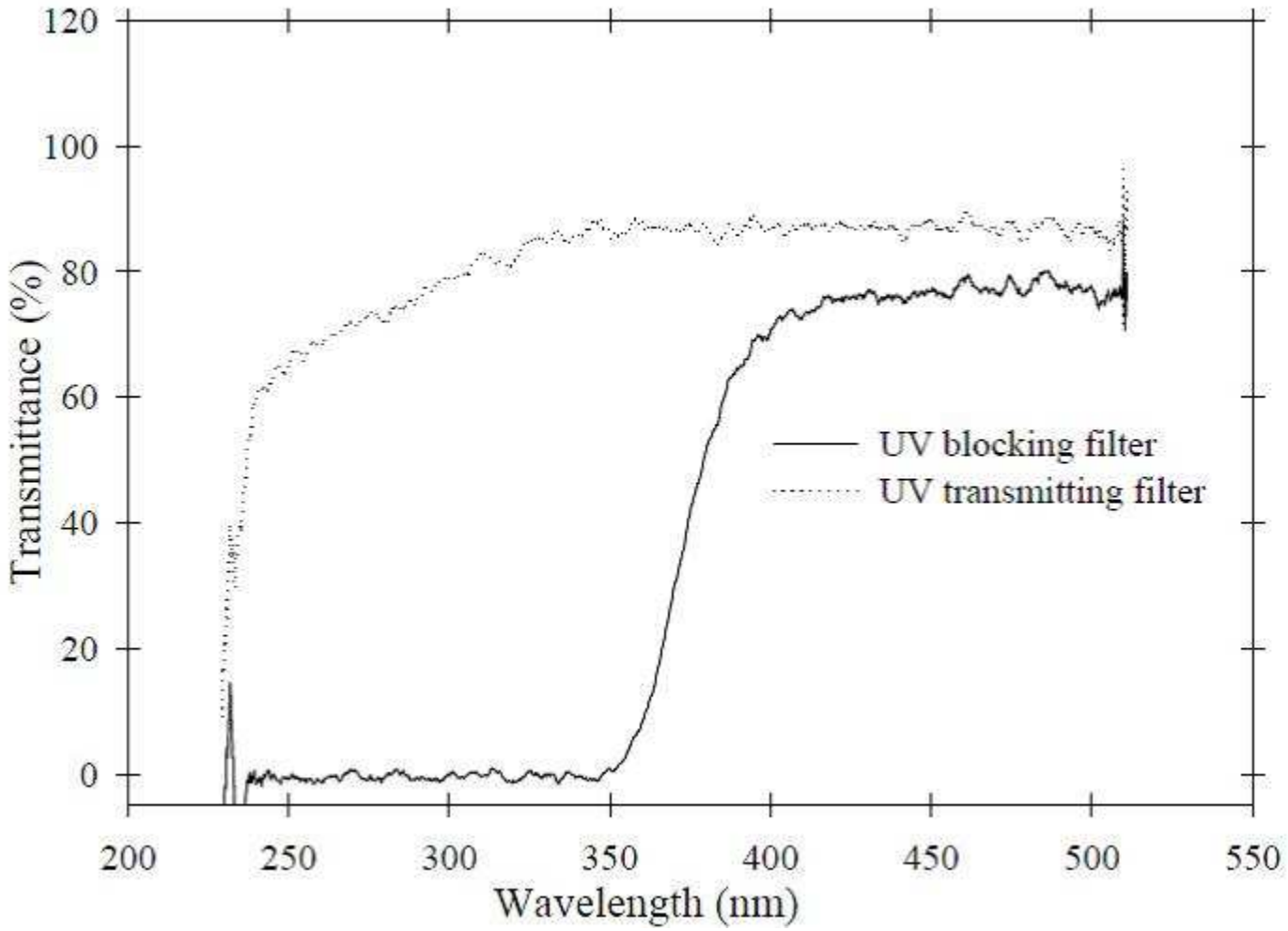

120
100
80
60
40
20
0
Transmittance (%)
200
250
300
350
400
450
500
550
Wavelength (nm)
UV blocking filter
UV transmitting filter

Fig 2

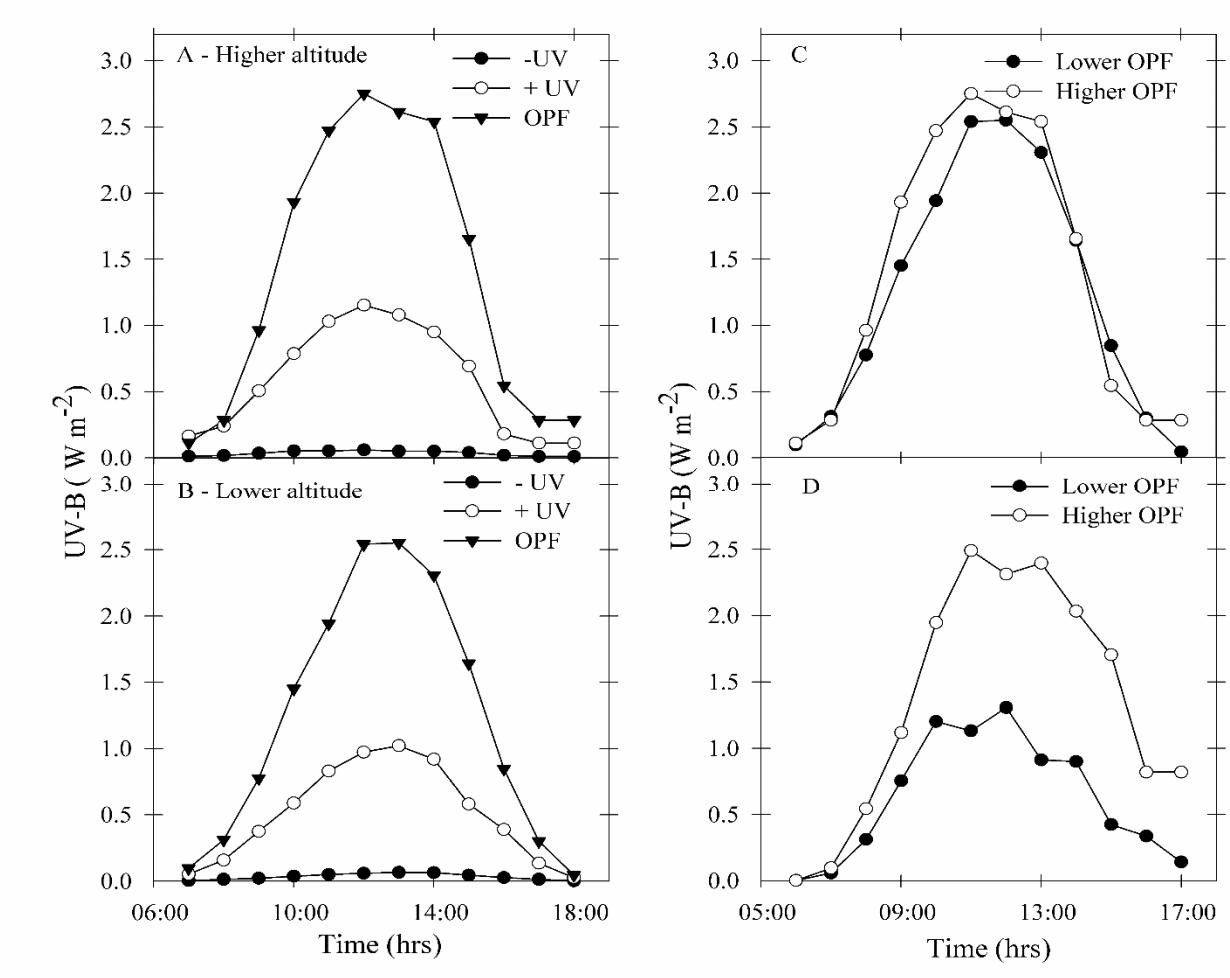
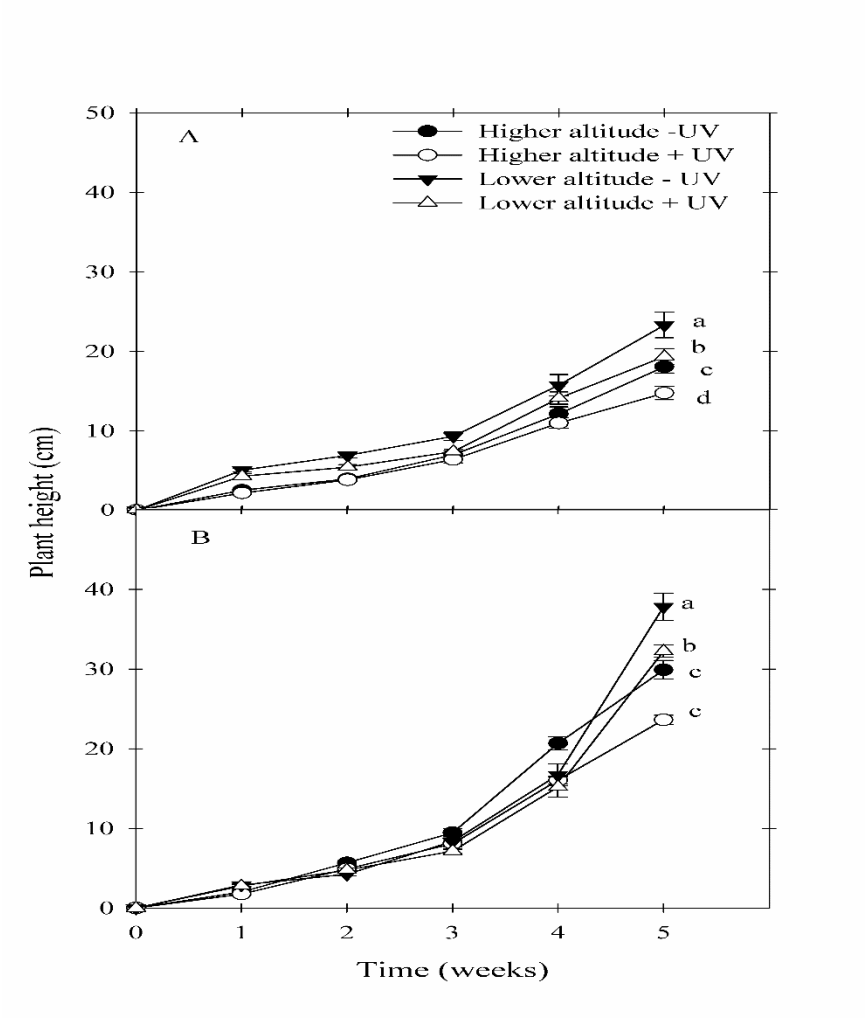

A - Higher altitude
-UV
+ UV
OPF
B - Lower altitude
- UV
+ UV
OPF
C
Lower OPF
Higher OPF
D
Lower OPF
Higher OPF
UV-B (W m-2)
UV-B (W m-2)
Time (hrs)
Time (hrs)
06:00
10:00
14:00
18:00
05:00
09:00
13:00
17:00
0.0
0.5
1.0
1.5
2.0
2.5
3.0

Fig 3.


A
B
Higher altitude -UV
Higher altitude + UV
Lower altitude - UV
Lower altitude + UV
a
b
c
d
a
b
c
c
Plant height (cm)
Time (weeks)
0
10
20
30
40
50
0
1
2
3
4
5

Paper - IV

Meseret Tesema Terfa, Amsalu Gobena Roro, Jorunn Elisabeth Olsen, Sissel Torre

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Effects of UV radiation on growth and postharvest characteristics of three pot rose cultivars grown at different altitudes

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ABSTRACT

The ultra violet (UV) radiation reaching the ground is classified as UV-B (315–280) and UV-A (315–400 nm) and the levels vary with altitude and latitude. Numerous studies have shown that UV-B has various effects on morphology, biochemical composition and molecular responses of different species. It is well known that the climate conditions during growth also affect how plants behave after harvest. However, less is known about the effect of UV radiation during growth on postharvest characteristics of ornamentals, and especially the role of UV-B. In this study we investigated the effect of natural levels of UV radiation at different altitudes (2794 m a.s.l. (high altitude) and 1700 m a.s.l. (low altitude)) on growth responses like morphology and flowering, postharvest water usage and shelf life of three pot rose cultivars ('Cygein', 'Snow White', 'Tom Tom'). Plants were grown under UV-transmitting or UV-blocking films at different altitudes. The results showed that UV radiation significantly reduced growth at both altitudes; however the effect was more prominent at lower altitude. Besides, higher level of solar UV radiation also delayed flowering by 7–10 days. Postharvest life and water usage were not significantly affected by UV radiation but rather by the altitude and plants produced at high altitude had a better control of water loss and a longer postharvest life compared to lower altitude-grown plants. In conclusion, UV radiation mainly affected morphology and development of the plants. However, stomata conductance, postharvest water usage and characteristics were rather affected by altitude differences than UV radiation.

1. Introduction

Ultraviolet radiation (UV) is a part of the non-ionizing region of the electromagnetic spectrum and comprises approximately 8–9% of the total solar radiation (Hollosy, 2002). UV is traditionally divided into three wavelength ranges: UV-C (200–280 nm) is extremely harmful to organisms, but not relevant under natural conditions of solar irradiation since it does not reach the ground due to efficient filtration by stratospheric ozone layers; UV-B (280–315 nm) represents only approximately 1.5% of the total spectrum, but is of particular interest since it can induce a variety of effects in plants; UV-A (315–400 nm) represents approximately 6.3% of the incoming solar radiation and is the least hazardous part of UV radiation (Hollosy, 2002).

UV-B has various effects on morphology, biochemical composition and molecular responses of different species. However, the responses depend on species, cultivar, experimental conditions, levels of UV-B and the interaction with other climate factors like temperature and photosynthetically active radiation (PAR) (Frohnmeyer and Staiger, 2003; Reddy et al., 2004; Brown et al., 2005; Berli et al., 2012). Even though UV-B effects on vegetative growth and morphology of plants are variable, reductions in shoot length and leaf expansion were found to be the most common effects (Mark et al., 1996; Caldwell et al., 2003; Zhao et al., 2003). Besides, extended exposure of plants to UV-B radiation results in higher accumulation of phenolic compound to absorb UV-B and reduce its penetration and cellular damage (Lois, 1994; Jansen et al., 1998; Caldwell et al., 2003). Accumulation of such secondary metabolites and reduction in leaf area are part of the strategy by which plants adapt and escape from harmful UV-B radiation, through reduction in its transmittance (Jansen et al., 1998).

Furthermore, there are many reports showing significant reduction in total plant biomass and photosynthetic capacity due to damages to the photosynthetic pigments and chloroplast structure (Teramura and Sullivan, 1994; Kakani et al., 2003), as well as inhibition of photosystem II (Ziska et al., 1993; Allen et al., 1997). Additionally, photosynthesis could be indirectly affected through reductions in stomata conductance (gs) (Day and Demchik (1996); Zeuthen et al., 1997). There have been contradictory results on the responses of UV-B regarding gs and stomata characteristics.

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0304-4238/

It has been indicated that elevated levels of UV-B radiation might decrease gas exchange through enhancement of stomata closure (Dai et al., 1995; Keiller and Holmes, 2001; Berli et al., 2012) but in some plants UV-B has also been shown to induce stomata opening (Musil and Wand, 1993).

Pre-harvest environmental conditions have an enormous effect on the shelf life of ornamentals like cut flowers, bedding plants and pot plants. Ornamentals are mainly grown in protected cultivation systems and the environmental conditions during growth such as light (Mortensen and Gislerød, 1999; Fjeld et al., 1994), day and night temperatures (Moe, 1975; Hamrick, 2003), carbon dioxide levels (Dole and Wilkins, 2005) and relative air humidity (Torre et al., 2001; Pettersen et al., 2007; Fanourakis et al., 2012) are all shown to affect the postharvest shelf life (for review see, Halevy and Mayak, 1979a, 1979b). Stomatal behavior and water relations are one of the main factors determining the potential postharvest life, especially for cut flowers, but also for some pot and bedding plants (Torre and Fjeld, 2001; van Doorn, 1997; Waterland et al., 2010a, 2010b). Studies have shown that the stomatal behavior in response to conditions of the cultivation environment, such as relative air humidity (Torre and Fjeld, 2001; Fanourakis et al., 2012), light quality (Terfa et al., 2012), and photoperiod (Mortensen and Gislerød, 1999), will persist also after harvest. Thus, the postharvest water relation might be dependent on the environment during growth.

UV-B can induce a range of specific plant responses, some of which are particularly desirable from a horticultural perspective. However, less is known about the effect of UV radiation during growth on postharvest characteristics of ornamentals, and especially the role of UV-B (280–315 nm). Although UV-B was earlier mainly considered a plant stressor and a potential source for damage, currently an ambient or ecological dose of UV-B is believed to be an important signal for plants rather than a stressor (Jansen et al., 1998; Searles et al., 2001; Jordan, 2002; Jenkins, 2009; Jansen et al., 2012). Novel technologies to manipulate UV levels are emerging. For example, by using different selective plastic films, either UV-blocking or UV-transparent, specific parts of the UV spectrum can be manipulated. This provides new opportunities in protected crop cultivation (Jansen et al., 2012).

Since UV-B at ground level varies with altitude and latitude, UV-B exposure of plants will depend on the specific growing site. Close to the equator commercial plant cultivation is possible also at high altitudes. For example, in Ethiopia highland areas have a mild climate for ornamental and other crops production. Ethiopia, is currently the second largest exporter of cut flowers in Africa (Gebreeyesus and Iizuka, 2012), and roses are produced in protected cultivation systems under plastic coverings but without heating. The two main locations where the commercial rose productions are intensively under way in Ethiopia are highlands (2400–2600 m a.s.l) around the capital, Addis Ababa, where the climate is characterized by high daily temperatures and cool nights, and Ziway (mainly characterized as lowland; 1100–1800 m a.s.l) where the temperatures are higher (25 °C in average). The UV radiation reaching the highland region of Ethiopia is higher compared to lowland due to the increase in solar UV radiation with altitude (Sullivan et al., 1992; Schmucki and Philipona, 2002). Obviously, there is also a huge difference in daily mean temperature and day and night temperatures between highland and lowland. However, the expected difference in UV-B at the two altitudes may also have a role in postharvest behavior either directly or indirectly by affecting stomata function and eventually postharvest water usage. In other postharvest study we have observed that there is a huge difference in postharvest life of different cultivars of roses grown at different altitudes, where plants grown at high altitude showed better postharvest characteristics as compared to low altitude grown ones (Terfa et al., unpublished result). Thus, the aim of this study was to test the role of natural levels of UV radiation at different altitudes in Ethiopia on growth responses like morphology and flowering, postharvest water usage and shelf life of different cultivars of pot-roses. These pot roses were grown under UV-transmitting and UV-blocking films at different altitudes.

2. Materials and methods

2.1. Study area and planting material

Field experiments covered with different plastic films (see below; Fig. 1) were carried out in the southern part of Ethiopia at two different locations commonly described as highland (Hagereselam) and lowland (Hawassa). Hawassa (7°3′N 38°28′ E) is located at an altitude of 1700 m a.s.l and Hagereselam (6°27′N 38°27′ E) at an altitude of 2794 m a.s.l. During the experiments climatic parameters at the experimental sites were recorded every hour by a thermo hygrometer data logger (Testo 174H, Testo comfort software basic, Version 5.0.2564.18771, Lenzkirch, Germany) hanged on the top of the plant canopy (Table 1). Three pot rose (*Rosa* × *hybrida*) cultivars collected from a commercial rose grower near Addis Ababa (Ethio Plants PLC, Alemgena, Ethiopia) were used in the experiments; 'Snow white' (white petals), 'Tom-Tom' (pink petals) and 'Cygein' (red petals).

2.2. Pre-cultivation and growth condition

Plants from the three pot rose cultivars were grown from a single node stem segment with one mature leaf. Cuttings were made from the middle and lower position of fully developed stems with open flowers and rooted in pots with coconut peat rooting medium (Galuku Lankaexport PVt. Ltd., Kurunegala, Sri Lanka) for 3 weeks. During the rooting the plants were kept under plastic cover to keep the air humidity high. After rooting the plants were transferred to a 15 cm new pot with fertilized coconut peat (Nitrogen–Phosphorus–Potassium (NPK) 12–7.5–28 ppm) and kept in shade house in Hawassa for about 10–12 days. The climate under the shade house was 20 °C ± 5 temperature, 70% relative humidity and 12/12 h of light/dark. Natural light was used during the experimental period. When the plants had 1–2 cm long shoots they were transferred to a structure made of UV-blocking plastic covers (selectively cut-off UV-B below 350 nm radiation; Solar EVA-5 High Diffuse opaque polyethylene film with 0.20 mm thick and 3 m wide, Revora plastic, The Netherlands), and UV-transmitting white polyethylene sheet (transmits all solar spectrum beyond 250 nm; 0.2 mm polyethylene sheet, Addis Ababa, Ethiopian) (Fig. 1).

The structure was 3 m × 3 m wide and 2 m high with the bottom and top sides (15 cm above ground and 15 cm below roof) left open to allow air ventilation. It was constructed in the North–South direction over the treatment plot to ensure the solar radiation reaching the plants only after passing through the filter as the sun moves from East to West. The main climate factors recorded inside the structure during growth were temperature, relative air humidity (RH), and UV-B distribution (Table 1 and Fig. 2). The photosynthetically active radiation (PAR) passing through the UV-blocking and UV-transmitting films was about 80% and 75%, respectively, compared with unfiltered radiation (Fig. 2). Hereafter plants growing under plastic film blocking UV-B and short UV-A radiation will be referred to as minus UV (−UV), and those grown under white transparent plastic film transmitting UV-B and UV-A radiation will be referred to as plus UV (+UV). The solar irradiance was measured using a PAR quantum sensor (Skye quantum sensor, Skye Instruments Ltd., Llandrindod Wells, UK), in ($\mu mol\ m^{-2}\ s^{-1}$). The amount of UV-A and UV-B were quantified by a UV-A and

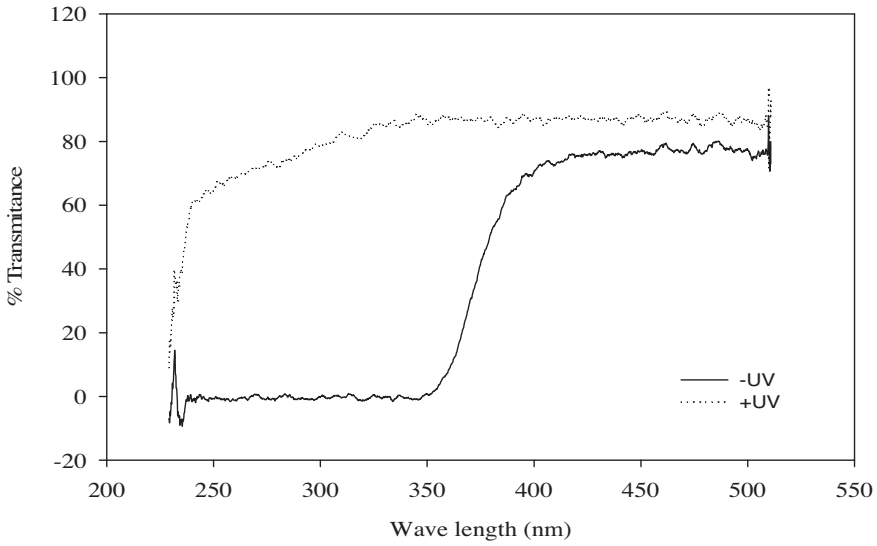

Fig. 1. Solar spectrum transmission of polyethylene films used in the growth experiment: UV-blocking polyethylene film (−UV) (solid line; blocks UV-B (280–315) and the short wavelengths of UV-A; Solar EVA-5 0.20 mm thick high diffuse opaque polyethylene film, Revora plastic, The Netherlands) and UV-transmitting polyethylene film (+UV) (dotted line; transmits all solar spectrum beyond 250 nm; 0.2 mm polyethylene sheet, Addis Ababa, Ethiopian).

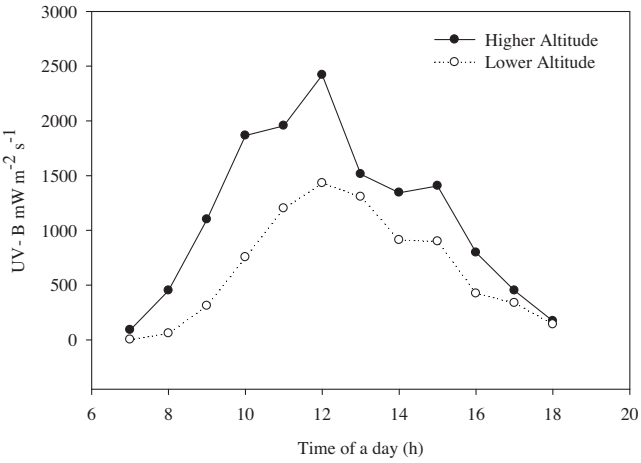

Fig. 2. UV-B distribution throughout the day at clear sky during the wet season (April–July, 2012) at higher altitude (solid line; 2794 m a.s.l, Hagereselam) and lower altitude (dotted line; 1700 m a.s.l, Hawassa).

UV-B Sensor (Skye UV-A and UV-B sensor, Skye Instruments Ltd., Llandrindod Wells, UK) in mW m^{-2} s^{-1}.

2.3. Growth parameter measurements

Plant growth parameters such as shoot length, average leaf area (LA), leaf number, leaf and shoot dry weight (DW) were analyzed when plants were at the commercial stage of sale with fully developed leaves and 1–3 open flowers. LA was measured with a LI-3100 leaf area meter (LI-COR, Inc., Lincoln, NE, USA). DW of the leaves and shoots was determined after drying the leaves and stems for 5 days at 70 °C. Twice a week flowering status was recorded in order to calculate number of days until open flower.

2.4. Stomata conductance and fluorescence

Stomata conductance (gs) was measured at local noon time (between 10:00 a.m. and 3:00 p.m.) on intact first fully expanded leaves of five plants per treatment in each experiment using an open system LCA-4 ADC portable infrared gas analyzer (Analytical development company, Hoddeson, England). During the measurements the calibrations/adjustment in the leaf cuvette and gas analyzer was: leaf surface area 2. 5 cm^2, ambient carbon dioxide concentration (C_{ref}) 340 μmol mol^{-1}, temperature of leaf chamber (T_{ch}) varied from 22 to 25 °C, leaf chamber molar gas flow rate (U)

Table 1
Climate data sampled during the experimental period (April–July, 2012) at both research sites: Higher altitude (2794 m a.s.l) and lower altitude (1700 m a.s.l). The temperature (T), relative air humidity (RH) and calculated water vapour pressure deficit (VPD) were sampled by a thermo hygrometer data logger hanged on the top of plant canopy inside each plastic film cover during the growing periods. While UV-B (mW m^{-2} s^{-1}), UV-A (mW m^{-2} s^{-1}) and PAR (μmol m^{-2} s^{-1}) were measured two times every hour on a clear sky day from 6 a.m. to 6 p.m. The climate data are the mean values of recordings from two experimental repeats.

Altitude	Plastic films	T_{mean} (°C)	RH_{mean} (%)	VPD (kPa)	PAR	UV-B	UV-A	UV-B/UV-A
High altitude	−UV	16.6b	76.7a	0.45	825.4a	35.5c	1722c	0.02c
	+UV	16.5b	77.7a	0.41	889.6a	885.4a	11970a	0.08a
Low altitude	−UV	24.6a	65.8b	1.12	599.8b	36.8c	1397c	0.03c
	+UV	25.3a	63.4b	1.16	675.8b	557.b	8612b	0.07b
p-Values								
Altitude		0.001	0.001	0.001	0.012	0.04	0.01	0.03
Film		0.973	0.845	0.082	0.15	0.01	0.001	0.01
Altitude × film		0.670	0.589	0.749	0.20	0.03	0.20	0.09

410 μmol s^{-1}, ambient pressure (p) 828 mbar and PAR (Q) at leaf surface was maximum up to 1500 μmol m^{-2} s^{-1}. The average leaf temperature during measurements varied between the locations. The leaf temperature for plants grown at the lower altitude varied between 30 and 32 °C while it was between 20 and 22 °C for plants at the higher altitude. Measurements were taken every 5 min for 30 min in each plant. The maximum efficiency of PSII photochemistry Fv/Fm was measured in the same time period by a plant efficiency analyzer Handy-*PEA* (Hansatech, Kings Lynn, UK).

2.5. Postharvest characters and measurements

To analyze postharvest characteristics six flowered rose plants with intact roots were transferred from each treatment to a common test room in Hawassa University. Plants were at the commercial stage of sale with fully developed leaves and 3–4 open flowers. The climate during testing were 58 ± 5% RH (corresponding vapour pressure deficit (VPD) was 1.2 kPa), irradiance 35 ± 5 μmol m^{-2} s^{-1} supplied by fluorescent tubes (Osram NAV T-400 W, Munich, Germany) as 12 h/12 h of light/dark, and a temperature of 23 ± 1.5 °C. Three control pots with no plants and only soil were also placed in the room to estimate the water loss through evaporation from the soil. All the pots were then weighed every day from the first day (D_0) until the end of the postharvest life duration for every plant. At the end of the postharvest life the leaf area of the plants was determined by a leaf area meter (LI-COR, LI-3100). Then rate of water loss (transpiration rate) per leaf area per day (H_2O cm^{-2} day^{-1}) was calculated. Assessment of the postharvest life duration was done visually according to a standard procedure (Association of Dutch Flower Auctions (VBN), 2005). The postharvest life of a plant was considered terminated when 50% of either one or more of the postharvest symptoms were visible. The visual symptoms taken into account were petal wilting, petal necrosis, leaf wilting and drying.

2.6. Statistical analysis

At both locations the experiment was repeated twice with the same experimental layout during the wet season (April–July, 2012). Since the trends of the results in the experiments were similar the data are presented as an average of the experimental repeats unless otherwise mentioned. Significant differences between means were tested for by applying normally distributed general linear models (GLM). Differences with $p \leq 0.05$ were considered significantly different. All statistical tests were performed in Minitab 16.1.1 (Minitab 16.1.1, windows version, State College, PA, USA).

3. Results

3.1. Plant growth and development

Number of days to flower opening was significantly affected by altitude and UV radiation in all cultivars. In general, plants grown at high altitude required 2–3 more weeks to get visible flower buds compared to low altitude (Table 2). Plants grown under −UV radiation flowered 7–10 days earlier in both altitudes as compared to +UV radiation (Table 2). There was no significant interaction between altitude and UV radiation in days to flowering. In addition, UV radiation caused petal blackening in the red color cultivar ('Cygein') and brown spots the petals on the white petal color cultivar ('Snow white').

The shoot length and leaf number were significantly affected by altitude and UV radiation in all the cultivars (Table 1). In all the cultivars higher altitude-grown plants had 9–10 cm longer shoots than those grown under lower altitude regardless of the UV radiation (Table 2). However, the internode number and number of leaves were 1.3 and 2 times higher respectively, in lower altitude than high altitude. UV radiation also significantly affected shoot length and number of leaves in all cultivars in both altitudes (Table 2). The reduction in shoot length and leaf number due to UV radiation was 25–35% and 15–19%, respectively, for all cultivars regardless of altitude. However, the reduction was more pronounced at low altitude and plants were on average 10% shorter than high altitudes plants in all cultivars (Table 2). Even though both altitude and UV radiation had a significant effect on shoot length, leaf number and internode number, the strongest reduction in all growth parameters was mainly due to altitude rather than UV radiation. There was a significant interaction between altitude and solar UV radiation on average leaf area (LA) and leaf dry weight (LDW) (Table 2). LA was reduced by 25–30% by +UV radiation, in both altitudes and

Table 2
Growth and morphology of *Rosa* × *hybrida* cultivars grown at different altitudes under different plastic coverings transmitting UV-A and UV-B (+UV) or blocking UV-B and short UV-A (−UV). Data are the mean values of measurements from two experimental repeats with ten replications in each ($n = 20$; $p < 0.05$).

Altitude	Plastic film	Cultivar	Shoot length	Leaf number	Internode number	Average leaf area	Leaf DW	Shoot DW	Days to flowering (weeks)
High altitude	+UV	'Cygein'	16.0	6.2	7.5	140.8	0.9	0.8	8.0
		'Tom-Tom'	19.9	5.7	7.8	218.4	1.7	0.9	7.5
		'Snow white'	16.8	5.0	6.3	98.6	0.8	0.6	8.0
	−UV	'Cygein'	21.7	9.3	7.0	252.3	1.9	1.8	6.5
		'Tom-Tom'	23.5	8.8	7.5	325.5	2.6	1.6	6.5
		'Snow white'	20.3	8.0	6.9	134.7	1.8	1.7	6.0
Low altitude	+UV	'Cygein'	6.7	13.2	9.0	67.0	0.6	0.4	4.0
		'Tom-Tom'	8.8	10.8	9.8	139.4	1.5	0.7	4.5
		'Snow white'	8.5	11.2	8.2	65.9	0.6	0.8	4.0
	−UV	'Cygein'	10.3	16.3	9.6	139.8	1.6	1.6	3.0
		'Tom-Tom'	12.4	12.8	9.5	177.4	2.4	1.4	3.5
		'Snow white'	11.5	14.8	8.8	90.7	1.7	1.4	3.0
p-Values									
Altitude			0.003	0.001	0.001	0.01	0.325	0.205	0.001
Film			0.001	0.001	0.621	0.01	0.021	0.001	0.002
Cultivar			0.042	0.032	0.050	0.001	0.011	0.052	0.356
Altitude × film			0.653	0.147	0.172	0.032	0.051	0.903	0.547
Altitude × cultivar			0.493	0.65	0.280	0.428	0.502	0.295	0.256
Film × cultivar			0.634	0.337	0.143	0.703	0.707	0.654	0.432
Film × cultivar × altitude			0.923	0.567	0.584	0.893	0.725	0.561	0.982

Table 3
Stomata conductance (gs) and Fv/Fm (maximal dark-adapted photosystem II efficiency) during growth of *Rosa* × *hybrida* cultivars grown under different plastic coverings transmitting UV-A and UV-B (+UV) or blocking UV-B and short UV-A (−UV) at different altitudes. Data are the mean values of measurements from two experimental repeats with five replications in each ($n = 10$; $p < 0.05$).

Altitude	Plastic film	Cultivar	Stomata conductance (mmol m^{-2} s^{-1})	Fv/Fm
High altitude	+UV	'Cygein'	149.7	0.79
		'Tom-Tom'	151.3	0.78
		'Snow white'	152.3	0.78
	−UV	'Cygein'	150.0	0.79
		'Tom-Tom'	152.0	0.79
		'Snow white'	154.0	0.79
Low altitude	+UV	'Cygein'	98.7	0.80
		'Tom-Tom'	96.7	0.81
		'Snow white'	92.3	0.80
	−UV	'Cygein'	100.0	0.81
		'Tom-Tom'	98.0	0.81
		'Snow white'	95.3	0.81
p-Values				
Altitude			0.014	0.052
Film			0.152	0.132
Cultivar			0.05	0.329
Altitude × film			0.703	0.654
Altitude × cultivar			0.283	0.206
Film × cultivar			0.769	0.908
Altitude × film × cultivar			0.823	0.709

the effect was more pronounced at low altitude. This was correlated with LDW, which was slightly affected by both altitude and UV radiation (Table 2).

3.2. Stomata conductance (gs) and fluorescence

Stomata conductance (gs) was significantly affected by altitude but not UV radiation and no interaction between altitude and UV radiation was found (Table 3). In general, plants (all cultivars) grown at high altitude had higher gs as compared to lower altitude during growth (Table 3; $p \leq 0.05$). The gs of plants were on average 1.8 times higher in high altitude as compared to lower altitude regardless of the UV radiation (Table 3). Fv/Fm (maximal dark-adapted photosystem II efficiency; indicates plant stress) was slightly affected by the altitude difference, where plants grown at high altitude showed a slightly lower average value of Fv/Fm (0.785) than those grown at lower altitude (Fv/Fm = 0.80) (Table 3). Fv/Fm was not affected by UV radiation in any of the cultivars.

3.3. Postharvest characters and water usage

Postharvest water usage was significantly higher in plants grown at low compared to high altitude; however the water usage was not affected by the UV radiation (Fig. 3). Plants grown at the lower altitude had twice as high water consumption than high altitude-grown plants (Fig. 3). There was also a significant difference in water consumption between cultivars; the cultivar Cygein used more water as compared to the other two cultivars at low altitude (Fig. 3). In line with this, in general, compared to lower altitude-grown plants, plants grown at high altitude had a longer postharvest life that also correlated with the postharvest symptoms recorded (Table 4). Postharvest symptoms such as petal wilting and leaf drying were more prominent in low altitude-grown plants than high altitude (Table 4).

4. Discussion

Under natural conditions plants are exposed to different levels of UV radiation, especially UV-B, depending on geographic location,

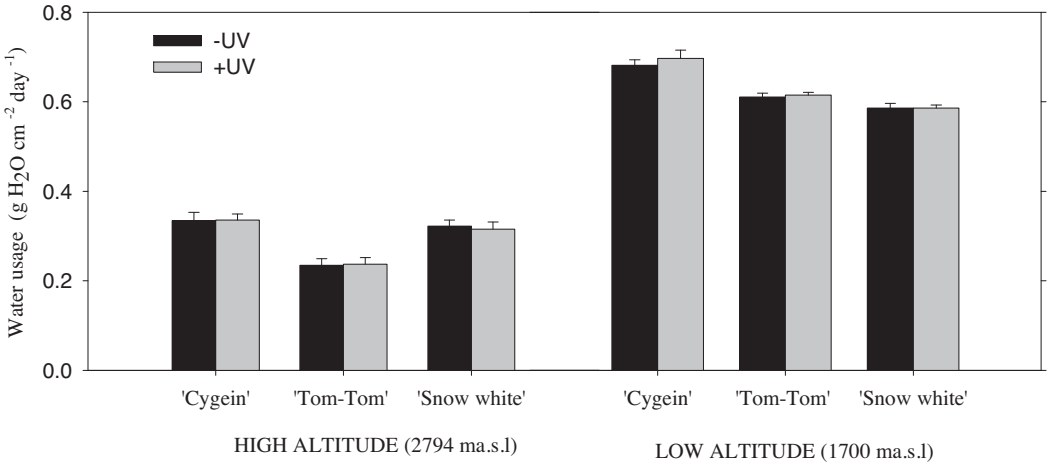

Fig. 3. Postharvest water usage of *Rosa* × *hybrida* cultivars grown under different plastic coverings transmitting UV-A and UV-B (+UV) or blocking UV-B and short UV-A (−UV) at different altitudes. The water usage was measured gravimetrically every morning until the end of the postharvest life after plants from different treatment were moved to a common postharvest room with 58 ± 5% RH (corresponding vapour pressure deficit (VPD) is 1.2 kPa), irradiance 35 ± 5 μmol m^{-2} s^{-1} supplied by fluorescent tubes as 12 h/12 h of light/dark, and a temperature of 23 ± 1.5 °C. The error bars indicate the mean values of measurements from two experimental repeats with six replications in each ($n = 12$).

Table 4
Postharvest characteristics of *Rosa* × *hybrida* cultivars grown at different altitudes under different plastic coverings; transmitting UV-A and UV-B (+UV) or blocking UV-B and short UV-A (−UV). Postharvest life terminated when 50% of the leaves or petals showed the mentioned symptoms individually or in combination. Data are the mean values of measurements from two experimental repeats with six replications in each ($n = 12$; $p < 0.05$).

Altitude	Plastic film	Cultivars	Petal wilting (% of total)	Leaf wilting (% of total)	Petal necrosis (% of total)	Leaf drying (% of total)	Postharvest life (days)
High altitude	+UV	'Cygein'	63.1	41.2	25.1	57	11.3
		'Tom-Tom'	62.2	43.1	29.2	60	13.5
		'Snow white'	64.7	40.4	32.8	61	12.5
	−UV	'Cygein'	62.5	40.2	23	58	12.2
		'Tom-Tom'	65.2	41.3	28	65	14
		'Snow white'	66.5	42	30	62	13
Low altitude	+UV	'Cygein'	71.2	56.8	37.2	69.4	8.5
		'Tom-Tom'	72.5	56.2	39.5	68.5	9.6
		'Snow white'	71.3	59.3	41.3	69.2	8.3
	−UV	'Cygein'	70.2	55.2	35.5	68.2	8.0
		'Tom-Tom'	73.5	56.1	38.1	66.3	9.1
		'Snow white'	70.5	57.2	39.5	67.8	8.3
p-Values							
Altitude			0.014	0.012	0.003	0.021	0.001
Film			0.452	0.132	0.329	0.536	0.324
Cultivar			0.245	0.329	0.042	0.482	0.568
Altitude × film			0.603	0.654	0.367	0.357	0.413
Altitude × cultivar			0.383	0.206	0.529	0.423	0.583
Film × cultivar			0.569	0.908	0.843	0.703	0.703
Altitude × film × cultivar			0.348	0.706	0.809	0.349	0.708

cloud cover, and solar altitude (Estupiñán et al., 1996; Rozema et al., 1997; Diffey, 2002). Even at the same geographical location and season the amount of UV-B reaching the ground varies with time of the day and time of the year and also depends on the interaction between UV-B and other climatic factors. In the present study we investigated the effect of UV radiation at different altitudes on growth, development and postharvest characteristics of pot roses. The UV-blocking film used in the experiment blocked all UV up to 350 (all UV-B and the short UV-A) while the +UV film transmitted the full UV range. Thus, the main difference between the two films is in the UV-B region (280–320) and the short UV-A (Fig. 1).

UV-B radiation is one of the key environmental signals that regulate plant responses including plant morphology (Jansen, 2002; Jenkins, 2009). In the present study, UV radiation affected most of the vegetative growth variables at both altitudes. A 30–40% reduction in shoot length and LA were found under the UV-transmitting film compared with the treatment where UV was blocked (Table 2). The reduction in shoot length and vegetative growth is a typical UV-B response found in many different species, e.g. such as lettuce, mung bean, maize, cucumber, grapevine and *Arabidopsis thaliana* (Krizek et al., 1997; Pal et al., 1997; Krizek et al., 1998; Jansen, 2002; Wargent et al., 2009; Berli et al., 2010, 2012). From this study, it is clear that all the rose cultivars tested responded similarly to UV radiation. The compact and shorter plants in +UV radiation were due to shorter internodes since the number of internodes was not affected by UV radiation (Table 2).

It has been demonstrated that LA is very sensitive growth parameters that easily respond to elevated UV-B due to reduced leaf formation and leaf expansion (Nogues et al., 1998; Zhao et al., 2003). Ballaré et al. (1995) and Grant (1999) also showed that when plants were exposed to UV-B, the LA was lower because of both smaller leaves and a lower number of leaves. These morphogenic responses are possibly a part of the photomorphogenic acclimatization mechanism of the plants to reduce the interception of the UV-B (Jansen, 2002; Jenkins, 2009). Besides, according to Hectors et al. (2010), UV treatment did not affect cell number, cell shape, cell area variation, or stomata formation, rather the reduction in leaf size was solely due to smaller pavement cells, because of impaired cell expansion at an early stage of leaf development.

Number of days to flower opening was significantly affected by altitude and solar UV radiation in all cultivars. The longest flowering time (2–3 weeks) was recorded at high altitude regardless of the UV radiation (Table 2). Even though flower induction in roses is autonomous flower development is promoted by increasing temperature and irradiance. Temperature is well known to facilitate flowering in many plant species (see review by van Doorn and van Meeteren, 2003). Shin et al. (2001) showed that in roses the number of days from bud break to flowering increased from 21.6 to 63.0 days as temperature decreased from 30 to 15 °C. The number of days to flower was primarily influenced by the temperature after formation of a visible bud. This suggests that the temperature after visible bud formation significantly affects the rate of flower development and opening. Plants grown at higher altitude experienced lower temperature during development and this might have delayed flowering. Furthermore, plants grown under −UV radiation flowered 7–10 days earlier in both altitudes as compared to +UV radiation (Table 2). However, the flower induction might have occurred earlier in +UV radiation since they had fewer number of leaves at flower opening. The delay in flowering might be an indirect effect of UV radiation, because of reduced leaf area resulting in lower light capturing and lower dry matter accumulation. Carbohydrates are essential to flowering of plants (Bernier et al., 1993) and an important energy source facilitating flower opening (Ho and Nichols, 1977; Marissen and La Brijn, 1995). In most species, the mobilization of storage carbohydrates and/or the import of sucrose is important in flower opening as flowering requires some energy (van Doorn and van Meeteren, 2003).

The effect of UV radiation on growth was more prominent at low altitude (with higher temperature) where the reduction in shoot length and LA was 10–15% higher than at high altitude, despite significantly higher UV-B level at high altitude (with lower temperature) (Tables 1 and 2). This might be due to the interaction of UV-B with other climatic factor such as temperature. Temperature is one of climate factors known to affect shoot elongation and cell expansion (Moe and Heins, 1990; Berghage and Heins, 1991). The interactive effect of temperature and UV-B has been shown to affect plant growth in many species (Mark and Tevini, 1996; Kakani et al., 2003; Reddy et al., 2004). However, some studies showed contradictory responses of these interactive effects. Nedunchezhian and Kulandaivelu (1996) showed that in cowpea plants, UV-B damage was greater for plants grown at 30 °C than for plants grown at 20 °C. In contrast, the UV-B induced reduction in seedling growth of maize and sun flower was alleviated by 4 °C increase in temperature from 28 °C to 32 °C (Mark and Tevini, 1996).

Plants from high altitude and high latitude ecosystems where UV-B and cold temperatures are naturally simultaneous or subsequent stresses, are less sensitive to enhanced UV-B than plants from low UV-B locations (van de Staaij et al., 1995; Binder and L'-Hirondelle, 1999; Chalker-Scott and Scott, 2004). This is partly due to increased tolerance towards UV-B as a result of low temperature. There are evidences about cross-tolerance between different stressors such as UV-B, low temperature and drought (Manetas et al., 1997; Chalker-Scott and Scott, 2004; Poulson et al., 2006), where plants showed increased tolerance against UV-B, low temperature or drought because of increased acclimation to the other stressor (Chalker-Scott and Scott, 2004; Poulson et al., 2006). The Fv/Fm values measured in our experiment show that UV radiation has no significant effect on Fv/Fm, and no indication of stressed plant. The values at both altitudes are within the Fv/Fm value range of 0.8 ± 0.05 shown for healthy and sun adapted leaves (Critchley, 1998) (Table 3).

Altitude rather than the UV radiation affected gs in all the cultivars (Table 3). In general, plants (all cultivars) grown at high altitude on average had 1.8 and 1.3 times higher gs as compared to at lower altitude, regardless of the UV radiation (Table 3). The gs was measured in the middle of the day when the temperature and irradiance reach their highest levels. The higher transpiration at high altitude and vice versa in low altitude might be due to effect of other climatic factors such as RH (VPD) and temperature. The VPD and temperature measured at the high altitude were lower than at the lower altitude (Table 1). The lower gs measured at low altitude might thus be due to the higher VPD and higher air and leaf temperature that increase the transpirational flux, forcing the plants to close their stomata in order to conserve water. Plants developed under higher VPD (low RH) are well known to have low gs during growth (Arve et al., 2012). Although plant surface area and density of stomata per leaf area are the major factors influencing the rate of water loss in plant, it has also been reported that gs is also related to altitude or difference in air pressure in addition to VPD (Smith and Geller, 1979; Leuschner, 2000; Gale, 2004; Körner, 2007). There are also reports indicating that with increasing altitude stomata density also increases, which positively correlates with increased gs (Holland and Richardson, 2009).

In the present experiment the postharvest water usage and postharvest life was significantly affected by altitude but not UV radiation (Table 4;). The water usage was related to the postharvest life and characteristics measured. Leaf wilting and leaf drying are typical postharvest characteristics for water stressed plants (Torre and Fjeld, 2001). Hence, plants grown at the lower altitude showed higher percentage of leaf drying and wilting which might be due to water stress because of high transpiration rate. This led to shorter postharvest life for lower altitude plants as compared to high altitude. Postharvest transpiration was higher for plants from lower altitude than high altitude. They had twice as high water usage than high altitude plants when transferred to postharvest room. Stomatal behavior and water relations are one of the main factors determining the potential postharvest life of cut flowers as well as for some bedding and pot plants (Waterland et al., 2010a,b). The postharvest water loss can be dependent on the stomatal behavior during growth (Torre et al., 2001; Fanourakis et al., 2012). It has been shown that environmental conditions during cultivation influence postharvest quality of roses by affecting the ability to control postharvest water loss (Halevy and Mayak, 1979a,b). For plants grown at high altitude, VPD (0.4 kPa) during growth was lower as compared to the lower altitude-grown plants (Table 1). However, when they were transferred to an environment where the VPD is very high (VPD in postharvest room: 1.2 kPa) they probably respond by closing their stomata to avoid water loss. However, for plants developed at the lower altitude, there was no significant change in VPD during growth (VPD: 1.12 kPa) and postharvest (VPD: 1.2 kPa). Since these plants did not sense any stimuli to close their stomata after transfer to the postharvest test room they continued to transpire as usual. Under natural conditions, plants are adapted to sudden environmental changes by physiologically adjusting themselves. This can be by dynamically controlling stomatal conductance; plants can effectively regulate long-distance water flow and water potential over short term which ultimately regulates stomata function (Hacke and Sauter, 1995; Laur and Hacke, 2013). Hence, in this experiment the ability of plants grown at high altitude to easily sense the changing environment and dynamically adapt to it by keeping their water balance and avoiding unnecessary water loss was a key factor for a better postharvest life.

In conclusion, UV radiation reduced shoot length and LA in both altitudes. However, stomata conductance, postharvest water usage and characteristics were rather affected by altitude differences than UV radiation. Hence, plants grown at higher altitude had a better control of water loss and a longer postharvest life than lower altitude-grown plants. UV radiation can induce a range of specific plant responses, some of which are particularly desirable from a horticultural perspective. However, from this particular study it is not recommended to use UV-transmitting plastic coverings during rose cultivation either at highland or lowland since it reduced the growth, increased discoloration of petals and delayed the flowering without improving the postharvest shelf life.

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Paper -V

Amsalu Gobena Roro and Admasu Tsegaye

Growth and morphology of pea (*Pisum sativum* cv. Oregon sugar pod II) grown under different shading screens in Ethiopian climatic conditions

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Abstract:

The use of different covering materials, like colored nets and films to shade or to manipulate light quality is increasing in areas with excessive light. Modifications of light have significant effects on growth and development of plants. In this study two types of imported screens (Svensson with strip ventilation, and white plastic) and one locally produced screen (yellow plastic) were used as greenhouse covers to study their effects on the performance and productivity of pea (*Pisum sativum* cv. Oregon sugar pod II) during the dry season in Ethiopia. Plants grown under the Svensson screen were 5.1–6.4 cm taller, had 2–3 more internodes and the internodes were 0.44–0.59 cm longer as compared to those grown under the yellow and the white plastic screens. However, no significant differences in dry matter or pod number were found between the screens. The difference in morphology was mainly due to the reduced transmittance of photosynthetic active radiation (PAR) and ultraviolet (UV) radiation of the Svensson screen as compared to the plastic screens (both white and yellow). Significantly smaller stomata aperture and lower leaf conductance were found on plants grown under yellow

plastic film as compared to the imported screens. Thus, plants grown under the locally produced yellow plastic film had 17% and 37% lower transpirational water loss as compared to the Svensson and the white plastic screens, respectively. Maximal PSII efficiency (Fv/Fm) was also lower in the locally produced yellow film as compared to the two imported screens, but Fv/Fm was not correlated with pod number. In conclusion, growth and development of pea are robust to changes in light climate. The cheap locally produced yellow plastic screen with relatively high PAR and UV transmittance is a suitable screen in the production of pea and an efficient tool to control transpirational water loss in warmer regions like Ethiopia.

Keywords:

Morphology

Pisum sativum

Plastic film

Covering material

Light spectrum transmittance

Pod

Screen

Stomata

Abbreviations: UV, ultraviolet radiation; Fv/Fm, maximal photosystem II efficiency; gs, stomata conductance; PAR, photosynthetically active radiation; RH, relative air humidity; masl, meter above sea level; LWR, leaf weight ratio; LAR, leaf area ratio; DAP, diammonium phosphate

1. Introduction

Solar radiation consists of different types of wavelengths ranging from the shortest wavelength, ultraviolet (UV), to the longest wavelengths, near infra-red (NIR). Light is the most important climate factor affecting growth and development of plants as an energy source for photosynthesis and as a signal controlling a wide range of processes. Photosynthetic active radiation, PAR (400–700 nm) is the spectral range which plants are able to use for photosynthesis. Different parts of the solar spectrum controls different processes like seed germination, flowering and morphology (Chory et al., 1996). Light, along with other environmental clues like temperature, enables plants to adapt and adjust their growth and morphology to the environment. However, the response and sensitivity to the quantity and the quality of light differ widely among plant species (Tinoco-Ojanguren & Pearcy, 1995). Shade-tolerant plants often have lower photosynthesis rates, and they are subjected to photoinhibition when exposed to strong sunlight, as compared to sun tolerant species (Öquist *et al.*, 1992; Demmig-Adams *et al.*, 1998; Zhang *et al.*, 2004; Aleric & Kirkman, 2005).

Light quantity and light quality can be manipulated to optimize plant production by adding light (Mortensen & Strømme, 1987; Olle & Viršile, 2013) or removing light and/or specific parts of the solar spectrum by the use of covering materials (Hemming et al., 2005; Krizek et al., 2005). The use of different covering materials, like colored nets and films to shade and/or to manipulate light quality is increasing in areas with excessive light; for example, near the equator. In addition to functioning as a method of providing shade (reduce PAR and temperature) and manipulating the light quality, the coverings are also used as a way to protect plants from diseases and pests (Antignus *et al.*, 1996; Díaz & Fereres, 2007). The response of a wide range of plants to a modified light environment created by colored films has been reported by different researchers (Li *et al.*, 2000; Li *et al.*, 2003). Some plant species tolerate

high PAR, but under extreme sunny and warm conditions high transmission of PAR may cause high leaf temperatures and photoinhibition (Yakovleva & Titlyanov, 2001). High leaf temperatures can induce flower and fruit abortion in different plant species (Aloni et al., 2001; Guilioni et al., 2003; Marcelis et al., 2004).

Modifications of the UV part of the light spectrum have significant effects on growth and morphology of plants (Kittas et al., 1999; Terfa et al., 2014). UV absorbing films are widely used as cover material in protected cultivation (Antignus et al., 1996; Elad, 1997). Some types of coverings transmit UV radiation and can have positive effects on plant quality (Luthria et al., 2006). However, the effects of these cover materials on crop behavior vary widely depending on species and cultivars (Mortensen & Strømme, 1987).

In Ethiopia, most of the ornamental crops and leguminous plants are growing under considerably warm and sunny climatic conditions. The greenhouse production system is a relatively new but increasing agriculture sub-sector in Ethiopia. The most common greenhouse type is a basic steel construction with a fixed or adjustable single roof vent or side vents. The constructs are covered with plastic films (mainly polyethylene) to decrease the light intensity for creating a cooler environment. The use of different types of colored filters and cover materials to regulate desired physiological and morphological responses in plants is a new agro-technological concept, and is of increasing interest in Ethiopia. There are different cover materials used but most common types are locally produced cheap plastic films. Other, more expensive types of shading materials like colored nets (Shahak et al., 2004) or shading materials with reflectors and open strips to allow ventilation by free airflow through the opening (Hemming et al., 2005) – have, to our knowledge, not been tested and compared with the locally produced plastic films commonly used in Ethiopia.

In this study three different covering materials were compared, one cheap locally produced plastic film (yellow), imported plastic film (white) and imported shading material

with strip ventilation (Svensson). The objective of this study was to assess growth and productivity of pea under the three different coverings and to evaluate their potential under Ethiopian growing conditions.

2. Materials and Methods

2.1. Experimental location and set-up

The field experiment was conducted in Hawassa, in the southern part of Ethiopia, during the dry season (January–April) 2013. Hawassa is located at 7°3′N 38°28′E and at an altitude of 1700 meters above sea level (masl). For the study three types of covering materials were used: (1) custom made Svensson shading screen (AB Ludving Svensson Bangatan 8,511 54 Kinna, Sweden), (2) white UV blocking plastic film (Solar EVA- 5 High diffuse opaque film with 0.20 mm thick, Rovero plastic (Krabbescheer-6 4941 VW Raamsdonksveer, The Netherlands) that selectively cut off solar spectrum below 350 nm , and (3) yellow plastic film (0.2 mm polyethylene sheet produced by the Ethioplastic company, Addis Ababa, Ethiopia). Plants grown under Svensson screening material will, hereafter, be referred to as “Svensson’’, those grown under white UV screening plastic film as “white”, and those grown under yellow plastic film as “yellow’’.

The shade structures were constructed from wooden frames having an area of 4 m^2 and a height of 2 m. In each structure, about 15 cm of open space was left uncovered below the roof and above the ground for air circulation. The structure was erected in the north–south direction over the treatment plot. This orientation ensured that solar radiation reached the plant only after passing through the filter as the sun moved from east to west.

The light spectrum transmittance of the two plastic films (Fig 1A), imported plastic film (White) and local plastic film (Yellow) were measured at Norwegian University of Life Sciences (NMBU) by illuminating the sample at the port of an integrating sphere (ISP-50-REFL Ocean Optics, Ocean Optics, Dunedin, Fla., USA) with 600 μm thick optical fiber and a DH2000 (Ocean Optics) halogen light source. The light transmitted into the sphere was measured with 400 μm fiber connected to an Ocean Optics SD2000 spectrometer. The direct light (Fig1B) was measured by the company Svensson with a spectroradiometer (LI-1800, L-Cor, USA). A SUN 1200 (Honle Germany) was used as a light source. Small samples of the coverings were placed over an integrated sphere connected to the spectroradiometer. The visible light range (400–700nm) was used to determine percent direct light under Svensson screening materials (Fig 1B).

2.2. Climatic data and measurement

Climatic parameters such as temperature and relative air humidity (RH) were sampled every hour in a 24 hour cycle on 12 selected days during the experimental period (77 days), by the use of mini data loggers (Testo 174H, Version 5.0.2564.18771, Lenzkirch, Germany). Each data logger was hung close to the plant canopy (1 m above the ground).The UV-B (W m^{-2}), PAR (μmol m^{-2} s^{-1}), and R:FR ratio were measured two times every hour from 06:00–18:00h, on four days, using Skye spectrosense2 (Skye Instruments Ltd, UK).

2.3. Pre-cultivation and experimental growth conditions

Seeds of pea (*Pisum sativum cv.* Oregon sugar pod II) were obtained from a commercial farm (Hadia flowers and vegetables farm, Addis Ababa, Ethiopia) and sown directly in pots (15 cm size) filled with coconut peat (Galuku Lanka Exports Pvt. Ltd., Sri Lanka) and fertilized with 28 ppm diammonium phosphate (DAP; $(NH_4)_2HPO_4$, 18%N, 46%P_2O_5), following the methodology of (Valenzuela, 1983). The pots were placed in a shade house prepared for seed germination. They were arranged in the shade house (25% shade) and subjected to similar environmental conditions – a temperature of 20 °C and 70% RH, with 12/12 hour light/dark during germination of the seeds. The photoperiod was 12 hrs. Six days after germination, when the shoots were 1–2 cm in length, 30 pots were transferred to each experimental plot covered with the different screens.

2.4. Plant material and growth analysis

2.4.1. Growth measurement of young plants

Non-destructive growth data such as leaf thickness, stomatal conductance, leaf surface temperature and chlorophyll fluorescence measurement were collected from 4–5 weeks old vegetative plants. Another group of plants (six plants per treatment) were used for destructive measurements like collection of imprints of leaf epidermis, leaf dry weight, stem dry weight, leaf weight ratio (LWR= total leaf dry weight/ dry weight of vegetative part), and specific leaf area (SLA=leaf area of single leaf/dry weight of single leaf) at the stage of 4–5 weeks age. For determination of SLA, single leaf area and dry weight, leaves were collected from the 4^{th} node of six plants in each treatment. Leaf area ratio (LAR= leaf area per plant/weight per plant) and

LWR were calculated based on the leaf area, and the above ground fresh weight and dry weight of each plant. Leaf thickness was measured with a digital vernier caliper on leaves from the 5th node.

2.4.1.1. Stomata parameters

Stomata number and morphology was measured on three fully expanded leaves harvested from 4th, 5th and 6th nodes of five plants during morning (10:00 to 11:00 hrs) time. To evaluate stomata morphology and features, epidermal imprints were made on the upper surface of fully expanded leaves by coating approximately a 1.5 cm x 1.5 cm area of the leaf surface with clear nail polish. After 10 minutes the painted area was covered with transparent 'sellotape'. The imprinted epidermis was immediately fixed to a glass microscope slide and samples were kept at the Horticulture laboratory (Awassa College of Agriculture, Ethiopia) until it was transported to Norway. At Norwegian University of Life Sciences (NMBU) the negative imprints were photographed using Leica DM5000 B microscope (Leica Microsystems, Buffalo Grove, Illinois, USA) at 40x magnification, Leica DFC425 digital camera (magnification 0.5x), and Leica application LAS V370. Stomata length was quantified by measuring longitudinally from end to end, stomata aperture was quantified by measuring the opening distance between the two guard cells, and the stomata area was determined by measuring the circumference of the stomata.

2.4.1.2. Chlorophyll fluorescence

Chlorophyll fluorescence was measured on fully expanded leaves (at the 4th, 5th and 6th nodes) from three plants in each treatment, during morning time (06:00–07:00 h), using a plant efficiency analyzer, Handy-*PEA* (Hansatech, Kings Lynn, UK), following the methodology of (Strasser et al., 2004). Measurements were taken from 4-week old plants. For maximal chlorophyll fluorescence emission, leaves were dark-adapted in the leaf clip for 15 min. Light was then provided by an array of three high-intensity light-emitting diodes at 1500 µmol m^{-2} s^{-1} to ensure that the photosynthesis was fully saturated during the measurements.

2.4.1.3. Stomata conductance and transpiration rate

Stomatal conductance (gs), leaf surface temperature and transpiration rate (mmol m^{-2} s^{-1}) were measured on fully expanded leaves of three plants (4-week old plants) at the 5th node, using an open system LCA-4 ADC portable infrared gas analyzer with leaf chamber PLC-4 (Analytical Development Company, Hoddeson, England). The transpiration rate was measured from the water vapor pressure of the air entering and leaving the leaf chamber. This measurement was taken from 12:00 to 13:00 h (local time) after 5 minutes, with the following specifications/adjustments: leaf surface area 6.25 cm^2, ambient carbon dioxide concentration 340 µmol mol^{-1}, temperature in leaf chamber varied from 34 to 47 ^oC, leaf chamber molar gas flow rate 410 µmols^{-1}, ambient pressure 828 mbar and PAR at leaf surface was maximum 1500 µmol m^{-2}. Three plants were selected from each treatment. In each plant a fully opened leaf (5th node) was used for stomata conductance, leaf surface temperature and transpiration rate measurement. Measurements were taken in each leaf every 5 minutes for 15 minutes.

2.4.2. Measurement of growth and flowering of pea

During 5 weeks of growth, parameters like plant height, leaf number, internode number and internode length were measured every 7th day. Plant height was measured with a ruler from the top surface of the pot to the shoot apical meristem until the first flower bud appeared. After flower initiation there was no further shoot elongation of the main stem. Leaf number was determined by counting fully opened leaves on each node of the main shoot. All internodes below the newly opened leaves were counted and measured to determine the number of internodes. Internode lengths of six plants from each treatment were determined by measuring the length between the nodes. The appearance of flowers was recorded every third day, starting from week five – when the first flower appeared – until the appearance of new flowers stopped (8th week).

2.4.3. Measurements of pod size and above ground biomass

Pod length and width were measured during pod development, beginning 4–6 days after flowering when the pods were < 0.5 cm. The length and width were measured every day until pod extension stopped (seed filling stage) (Ohyama, 1983). Pod length (longitudinal section) and width (horizontal section) were analyzed at seed filling stage (about 15–20 days after flowering). The lengths of the pods were measured longitudinally, following the curvature of the pod. Pod width was measured at the middle of the pod length. During harvesting the total number of pods per plant, as well as the total and the individual pod fresh weights were determined. Leaves and stem of each harvested plant were separated, and fresh weights of stem and leaves were measured. Leaf area per plant was measured with an LI-3100 leaf area meter

(LI-COR, Inc., Lincoln, Nebraska, USA). Dry weight of above-ground biomass was measured after drying at 70 ºC for 72 hours.

3. Statistical analysis

Significant differences between means were tested using one-way analysis of variance (ANOVA) and Tukey's test with $p \leq 0.05$ significance level. Average values for each plant were used in the analysis. Data were checked for equal variance before ANOVA analysis. All statistical tests were performed in Minitab 16.1.1 (Minitab 16.1.1, Windows version, State College, Pennsylvania, USA). The experiment was done only once.

4. Results

4.1. Climate data and measurement

The light conditions and temperature measured under each screen material are presented in Table 1, and Fig 3. Although a big difference in mean temperature was not observed, the temperature under the yellow plastic cover seems higher by 1–2 ℃ during the middle of the day (12:00–14:00 local time) as compared to the Svensson and the white covering materials (Fig 3). However, the Svensson covering material had 55% and 43% less PAR than the locally produced yellow plastic shading material and the imported white plastic cover material, respectively (Table 1). However, the latter two had almost the same PAR levels. Moreover, the ratio of red to far red light (660/730 nm) was slightly higher under locally produced plastic cover material than under the two imported covering materials. The lowest R/FR ratio was measured under the Svensson covering material (Table 1). The UV-B level was only 4% under

the white plastic covering material, as compared to 17% and 23 %, respectively, under the Svensson and the local covering material. (Table 1).

4.2. Morphology of young plants

4.2.1. Leaf traits , stomata aperture and stomata area

Leaf area ratio (LAR) was 14% and 16% higher for leaves developed under the Svensson screen, as compared to the white and the yellow covering materials, respectively. However, leaf thickness, specific leaf area (SLA) and leaf weight ratio (LWR) were not significantly different between the coverings (Table 2). Smaller stomata aperture was found for plants produced under the yellow film, as compared to the white plastic and the Svensson screen. A similar trend was found in stomata area (Table 3). However, no significant difference in stomata number was found between the treatments (Table 3). As in the case of stomata aperture, stomatal conductance and transpiration rate were significantly reduced under the yellow covering material, as compared to the white covering material and the Svensson screen (Table 4). Leaf surface temperature was not significantly different ($p>0.05$; Table 4).

4.2.2. Chlorophyll fluorescence

Plants grown under the Svensson screen had higher maximal photosystem II efficiency (Fv/Fm) than plants grown under the white and the yellow covering materials. The lowest Fv/Fm value was measured in plants grown under the local yellow plastic (Table 4).

5. Measurement of morphology and yield

Plants grown under the Svensson covering material were 5.1 and 6.4 cm taller than plants grown under the white and the yellow coverings, respectively (Fig 2). Plants produced under the Svensson covering material had 2–3 more internodes and 0.44 to 0.59 cm longer individual internodes than plants produced under the white and the yellow covering materials (Table 5).

No differences were observed in flowering time between plants grown under the different covering materials. All the plants flowered after five weeks (data not shown). Moreover, all covering materials had similar effects on leaf area, leaf number and flower number during the growing period (Table 5). The number of pods, pod length, pod width, number of seeds per pod, as well as pod fresh weight per plant and individual pod fresh weight, were similar and no significant differences were found among the covering materials ($p>0.05$, Table 6). Further, no significant differences in dry matter accumulation and distribution were found between the treatments (Table 7).

6. Discussion

The purpose of this study was to compare three different covering materials and evaluate their effects on growth and development of pea in Ethiopian climate. Since Svensson covering is a ventilated reflective screen with lower light transmission, a lower leaf temperature was expected as compared to the two plastic films. However, no significant differences in leaf temperatures inside the small (4 m^2) greenhouses were found (Table 1).

The main difference between the Svensson covering and the plastic films was lower PAR transmittance in the case of the former (Table 1). For the Svensson screen there is a high transmission in the lab, whereas the transmission in the field is much more reduced (Fig. 1B). This difference can be explained by dust reducing the transmission and by the "tent" effect. The "tent" effect means that much of the light transmitted through the Svensson screen is diffused light. This diffused light will be spread in all directions over a much larger area than the roof area. Therefore, the light reaching the plants will be much attenuated. This "tent" effect will be much greater in these small experimental tents than in tents with a large roof area. For the two other clear screening materials more of the light transmitted is direct light and less diffuse light. The tent effect will be smaller and the difference between lab measurements and field measurements of light transmission will be less (Fig. 1).

Also, R/FR ratio was slightly lower under the Svensson covering, as compared to the plastic films (Table 1). Plant morphology and productivity are commonly influenced by environmental factors such as light, temperature and air humidity (Eskins, 1992; Jansen et al., 1998; Mortensen, 2000). However, the differences in productivity were found to be rather small in this study. The dry matter accumulation of the plants and the number of pods were almost the same under the three coverings, and no significant difference was found in pod size or fresh

weight per pod (Table 6 and 7). This shows that the pea plant is robust to changes in light climate.

In this study the plants were more elongated under the Svensson screen. It is likely that the reduced irradiance and the lower R:FR ratio in the Svensson covering material are the reasons for the growth stimulation, as compared to the plastic film (Table 1, Fig 1). Moreover, in addition to longer internodes, the plants grown under the Svensson screen had significantly more internodes – indicating that the growth rate (leaf/day) must have been higher in plants developed under the Svensson screen, as compared to the two plastic films. Pea is a fast growing type of vine crop that requires support to hold the plants uprights as they grow taller. In a commercial production system, dwarf varieties that only grow 30-60 cm in height might be optimal without additional support from staking or trellis material. Dwarf plants are also strong enough to self-support and keep their pods away from the soil surface (Powell & Marks, 2003; Tsado, 2012). In this study, the results showed that all coverings resulted in rather short plants (< 40 cm).

Plants under the Svensson covering material had 16% higher LAR than plants grown under the yellow plastic material. Poorter and Remkes (1990) reported that fast growing plants have a higher LAR, which is the fraction of total plant weight allocated to leaf area, than slow growing plants. Moreover, others have indicated that shaded plants have a higher biomass allocation to leaves, and a higher leaf area per unit leaf mass, resulting in a higher leaf area ratio (Popma & Bongers, 1988; Osunkoya *et al.*, 1994). Our result confirmed that, plants grown under a lower irradiance, like those under the Svensson covering, had higher LAR than plants growing under higher irradiance (Tables 1 and 2).

The yellow covering material induced a significant reduction in stomatal aperture and stomatal conductance. However, there was no significant difference between the Svensson

screen and the white plastic film (Tables 3 and 4). The reduction in stomatal aperture and conductance resulted in reduced transpiration under the yellow covering material, probably because of the higher PAR and slightly higher UV-B. Previous studies also reported that exposure to UV-B radiation significantly reduces stomata density and opening in UV-B sensitive cultivars (Dai *et al.*, 1992; Jansen & Van Den Noort, 2000). We did not find differences in stomata number between the different coverings. However, we observed that higher UV-B and PAR under the yellow material reduced the stomata conductance by 34% and transpiration rate by 17%, as compared to plants grown under the Svensson covering material. In one study (Tossi et al., 2014), it was found that higher UV-B fluence rate strongly reduced stomata aperture and conductance in *Arabidopsis.*

Pea plants have rather shallow rooting depth (rarely exceeding 100–120 cm) as compared to barley (*Horeum vulgare* L.), wheat (*Tritium aestivum*) and lupin (*Lupines angusifolious* L.) in a similar soil type (Hamblin & Hamblin, 1985; Hamblin & Tennant, 1987; Andersen & Aremu, 1991; Hauggaard-Nielsen *et al.*, 2001). These reports suggested that shallow root distribution may lead to late season water deficits. Agronomical techniques to manipulate the morphology and physiology of plants to reduce water usage, may help shallow rooted crops like pea to grow and produce optimum yield under water stress conditions. Reduction in plant size and leaf area, promoting early flowering and minimizing stomata conductance, are opportunities to manipulate water use efficiency against plant productivity (Blum, 2005).

Several studies clearly show that plants vary greatly in their response to ambient UV-B radiation. In some species enhanced UV-B radiation inhibited growth but in others it stimulated growth (Adamse et al., 1997; Krizek et al., 1997; Pal et al., 1997). Different reports also show that plants grown under high UV-B radiation suffer chlorophyll damage, which

could be due to its direct absorption of UV-B (Lingakumar & Kulandaivelu, 1993) or due to inhibition in the Chl biosynthesis (El-Mansy & Salisbury, 1971). However, this was not the case in our experiments. The chlorophyll content in pea was not significantly affected by the type of screen (data not presented). Removal of UV-B from the growth environment has been a common strategy to avoid UV related stress in plants. In our experiment the lowest Fv/Fm value was recorded on pea plants grown under the locally produced yellow covering material. Plants grown under the yellow plastic also received the highest level of PAR and UV radiation, compared to the other two imported covering materials. In many respects, plants grow as well under the Svensson screen as under the two other screens, although the light level is about half under the former as compared to the latter (Table 1).These results indicate that photosynthesis is already saturated at about 600 $\mu mol\ m^{-2}\ s^{-1}$.

Therefore, a doubling of the light level will not result in increased growth. This explanation is supported by the dawn measurements of Fv/Fm. Maximal PSII efficiency values of 0.77 and 0.79 in the morning, compared with 0.83 for plants under the Svensson screen, indicate that the plants under the two plastic films have not recovered fully from photoinhibition caused by excess light the previous day (Table 4). The high Fv/Fm value (0.83) for plants grown under the Svensson screen indicates that these plants are not stressed by high irradiance. Values close to 0.83 indicate unstressed plants (Baker, 2008).

Overall, however, only small differences were found between the more expensive imported white film and the less expensive locally produced covering; no differences were found in yield and pod quality. The locally produced yellow plastic cover might, therefore, be recommended for pea production in Ethiopian climate. However, the stability of the plastic covers was not tested in this study. Some films degrade easily in high light intensities and this is also an important quality parameter to evaluate.

7. Conclusion

The present study shows that the pea cultivar used in this study is robust to changes in light climate. Pea grown under the cheap locally produced yellow plastic screen produced similar number of pods to that grown under the more expensive imported screening material tested in this study. The higher transmission of PAR and UV-B through the yellow plastic film significantly reduced plant height, internode number, internode length and Fv/Fm, as compared to the imported Svensson screen, but the changes did not affect the yield. Lower stomata aperture and leaf conductance were measured under the yellow screen and resulted in reduced transpiration rate, as compared to the imported screens. Thus, the yellow screens can be efficient in reducing the water consumption in pea production. However, cost benefit analysis and the quality of the plastic (e.g. its stability) needs to be studied further.

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Fig. 1. Light transmittance through (A) imported plastic film Solar EVA- 5 High diffuse opaque film 0.20 mm thick (White) and locally produced 0.20 mm polyethylene sheet produced by Ethioplastic Company, Addis Ababa (Yellow) and (B) imported custom made shading screen (Svensson).

Fig. 2. Plant height was measured for pea plants grown under imported covering material (Svensson and white) and locally produced covering material (yellow) at Hawassa (1700 masl) in Ethiopia during the dry (January–April) season. Values are the mean of six plants ±SE.

Fig. 3**.** Temperature data was collected during the experimental period (January–April, 2013) at Hawassa (1700 masl) under imported covering materials (Svensson and white plastic) and locally produced covering material (yellow plastic). The temperature was sampled using a mini data logger, hung on the top of the plant canopy inside each covering material, during the experimental period (77 days). Data were measured every hour in each treatment for 12 days. Each point represents the average value of 12 measurements.

Table 1: Ambient irradiance levels and irradiance levels of UV-B (W m^{-2}) and photosynthetic active radiation (PAR) (µmol m^{-2} s^{-1}) and R:FR ratio below Svensson, white and yellow screens were measured in the middle of the day (11:30-14:30) at Hawassa in Ethiopia, during the dry (January–April) season of the year 2013. Percent reduction in irradiance below the screen, compared with ambient irradiance levels is also shown. R:FR ratios were measured two times every hour from 11:30–14:30 on four days. Data in the R:FR is the mean value ± SE, (n=4)

Screens	UV-B Ambient (W m^{-2} s^{-1})	UV-B below screen (W m^{-2} s^{-1})	% UV-B reduction	PAR Ambient (µmol m^{-2} s^{-1})	PAR below screen (µmol m^{-2} s^{-1})	% PAR reduction	R:FR ratio
Svensson	1.8	0.3	85	2000	612	70	0.95±0.01c*
White	1.8	0.08	96	2000	1083	46	1.0±0.01b
Yellow	1.8	0.43	77	2000	1372	31	1.11±0.00a

* Different letters in the R:RF ratio column indicate statistically significant difference at $p \leq 0.05$, Tukey's test

Table 2: The table shows the impact of different covering materials (such as imported Svensson and white plastic, and locally produced yellow plastic) on pea leaf parameters grown at Hawassa (1700 masl) during the dry season (January–April) 2013 for 4–5 weeks old plants. Values are mean values ±SE, (n= 6).

Leaf parameters	Covering materials		
	Svensson	White	Yellow
Leaf thickness (mm)	0.60±0.06a*	0.62±0.12a	0.67±0.15a
SLA ($cm^2\ g^{-1}$ DW)	366.42±16.8a	375.40±61.1a	303.9±31.4a
LAR ($cm^2\ g^{-1}$)	30.25±1.26a	25.92±0.70b	25.35±0.67b
LWR (g DW g DW^{-1})	0.54±0.02a	0.51±0.01a	0.53±0.01a

* Different letters in the same row indicate statistically significant difference at $p \leq 0.05$, Tukey's test

Table 3: The table shows the stomata number and stomata size in pea leaves grown under imported covering materials (Svensson and white plastic) and locally produced covering material (yellow plastic) at Hawassa (1700 masl) during the dry season (January–April). Five leaf samples were used to estimate stomata number and morphology. From each leaf sample, ten stomata were used to calculate stomata length, stomata aperture and stomata area. The values are mean values ± SE, n=50.

Stomata parameters	Covering materials		
	Svensson	White	Yellow
Stomata number	12.0±1.26a*	14.0±1.0a	13.0±1.41a
Stomata length (µm)	19.52±0.90a	19.66±1.25a	17.65±1.19a
Stomata aperture (µm)	6.53±0.94a	6.13±0.73a	3.4±0.34b
Stomata area (μm^2)	199.92±8.85a	192.74±15.32ab	144.53±16.3b

* Different letters in the same row indicate statistically significant difference at $p \leq 0.05$, Tukey's test

Table 4: The table shows the impact of different types of covering material (imported Svensson and white plastic, and locally produced yellow plastic) on stomata conductance and transpiration rate. Leaf surface temperature, chlorophyll content and Fv/Fm were measured in pea (*Pisum sativum* cv. Oregon sugar pod II) leaves of 4 week old plants during dry season (January–April 2013) at Hawassa (1700 masl). Stomata conductance, transpiration rate and leaf temperature were measured five times for each of three fully expanded leaves (average used in statistical analysis) from each of three plants (n=3). Three samples for chlorophyll were analyzed for one combined sample from each treatment (n=1). Measurements of Fv/Fm were taken from three fully expanded leaves from each of three plants (n=9). The values show mean ± SE.

Parameters	Covering materials		
	Svensson	White	Yellow
Stomata conductance (mmol m^{-2} s^{-1})	0.067±0.03a*	0.077±0.016a	0.044±0.003a
Transpiration rate (mmol m^{-2} s^{-1})	3.34±0.27b	4.4±0.28a	2.77±0.09b
Leaf surface temperature (℃)	34.18±0.58a	34.48±0.36a	35.18±0.12a
Fv/Fm	0.83±0.009a	0.79±0.005b	0.77±0.009c

* Different letters in the same row indicate statistically significant difference at $p \leq 0.05$, Tukey's test

Table 5: This table shows the effects of different covering materials (imported types – Svensson and white plastic – and local yellow plastic) on the growth and morphology of plants grown during the dry season (January–April) of 2013 at Hawassa (1700 masl). Leaf number, internode number and internode length were recorded from six plants every seven days. However, total leaf area was measured at week five, when the plants showed the first flower bud. The data are the mean values of measurements from six plants in one counting (Mean ± SE, n= 6).

Growth parameters	Covering materials		
	Svensson	White	Yellow
Leaf number	23.7±2.69a*	20.7±2.20a	26.0±2.80a
Leaf area (cm^2)	555.3±93.9a	461.4±41.6a	545.7±34.4a
Internode number	16.17±0.60a	15.33±0.67ab	13.00±0.68b
Internode length (cm)	3.4±0.14a	2.96±0.12ab	2.81±0.2b
Flower number	12.3±0.97a	11.00±1.19a	13.8±0.51a

* Different letters in the same row indicate statistically significant difference at $p \leq 0.05$, Tukey's test

Table 6: This table shows the productivity of pea plants grown under different covering materials (imported type Svensson and white plastic and locally produced yellow plastic) at Hawassa (1700 masl), Ethiopia, during the dry season (January–April) in 2013. The values are the mean ±SE of six plants.

Yield parameters	Covering materials		
	Svensson	White	Yellow
Number of pods plant^{-1}	6.67±0.92a*	6.67±1.36a	7.33±1.41a
Pod length (cm)	6.71±0.20a	5.87±0.18a	6.47±0.30a
Number of seeds pod^{-1}	4.4±0.19a	4.2±0.15a	4.5±0.25a
Pod width (cm)	1.92±0.08a	1.84±0.10a	1.90±0.10a
Fresh weight of pods plant^{-1}	14.89±1.66a	13.88±4.47a	15.76±2.32a
Fresh wt. per pod (g)	2.42±0.41a	1.89±0.322a	2.3±0.233a

* Different letters in the same row indicate statistically significant difference at $p \leq 0.05$, Tukey's test

Table 7: The table shows the dry matter distribution of pea plants grown under imported covering materials (Svensson and white plastic) and locally produced covering material (yellow plastic) at Hawassa (1700 masl) during the dry season (January–April) in 2013. The data shown are the mean values of measurements from six plants in one counting (Mean ± SE; n= 6). Values in parentheses indicate the proportion of dry matter allocated to different plant parts.

Parameters	Covering materials		
	Svensson	White	Yellow
Total dry weight (g)	7.14±0.42a*	7.79±0.38a	8.00±0.44a
Leaf dry weight (g)	1.16±0.26a (16.25%)	1.11±0.11a (14.25%)	1.29±0.06 (16.13%)
Stem dry weight (g)	0.95±0.16a (13.3%)	1.07±0.13a (13.74%)	1.17±0.1a (14.63%)
Pod cover dry weight (g)	0.63±0.05a (8.82%)	0.65±0.23a (8.34%)	0.74±0.12a (9.25%)
Seed dry weight (g)	4.41±0.28a (61.76%)	4.95±0.33a (63.54%)	4.79±0.38a (59.88%)

* Different letters in the same row indicate statistically significant difference at $p \leq 0.05$, Tukey's test

Fig.1

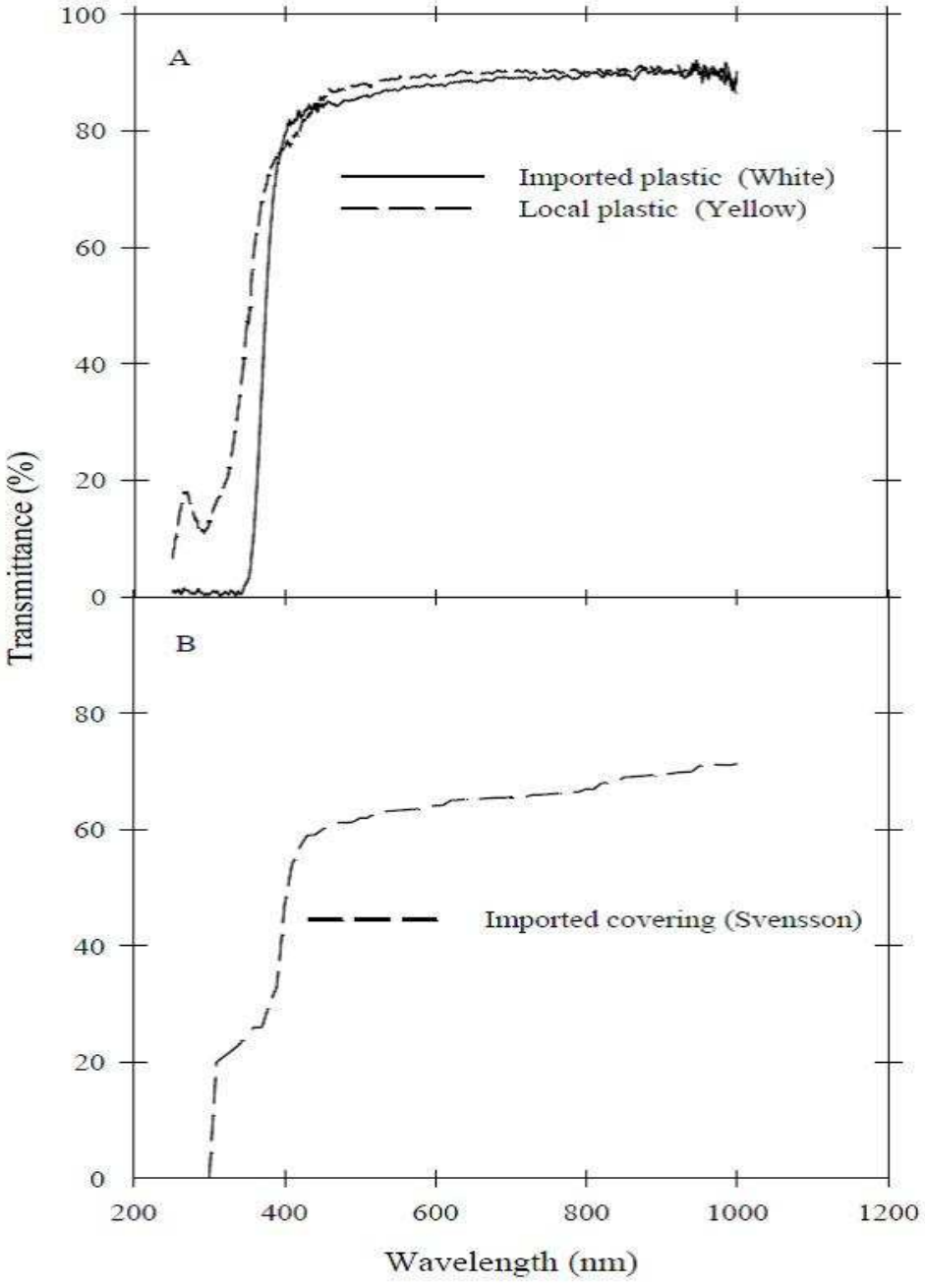

A
B
Imported plastic (White)
Local plastic (Yellow)
Imported covering (Svensson)
Transmittance (%)
Wavelength (nm)
0
20
40
60
80
100
200
400
600
800
1000
1200

Fig.2

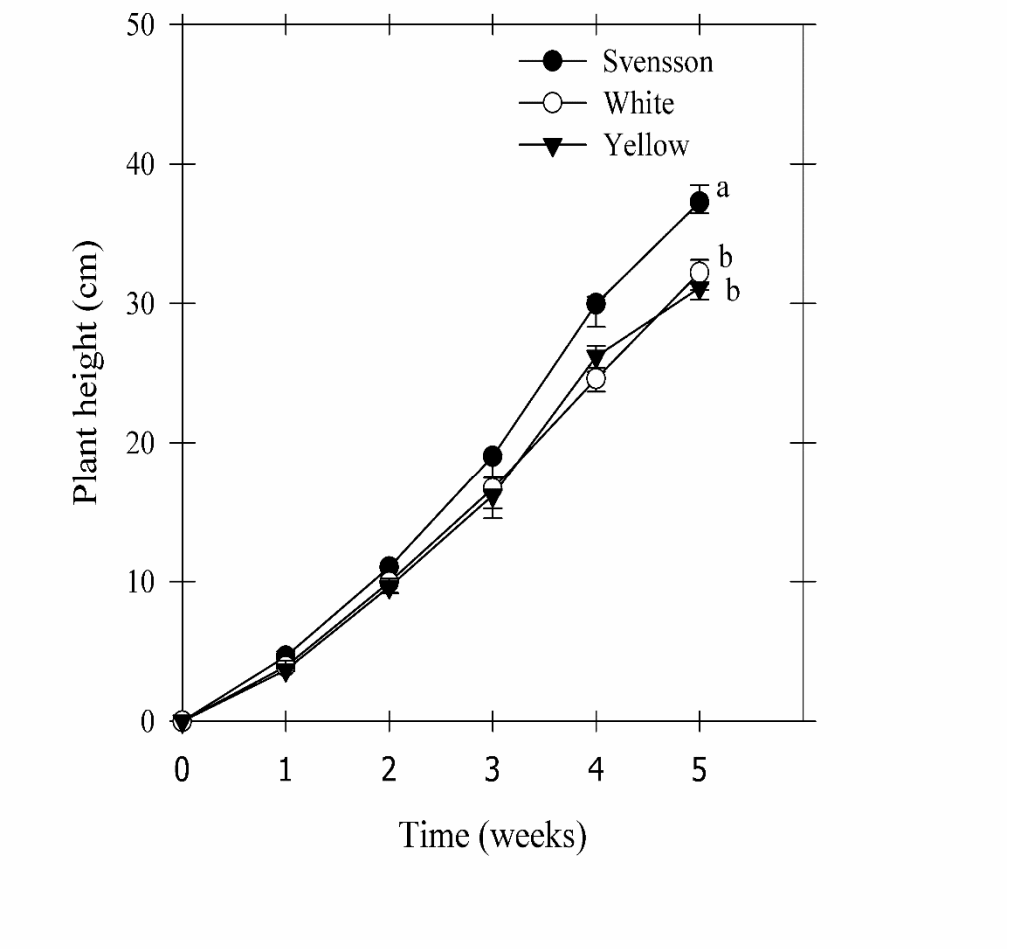
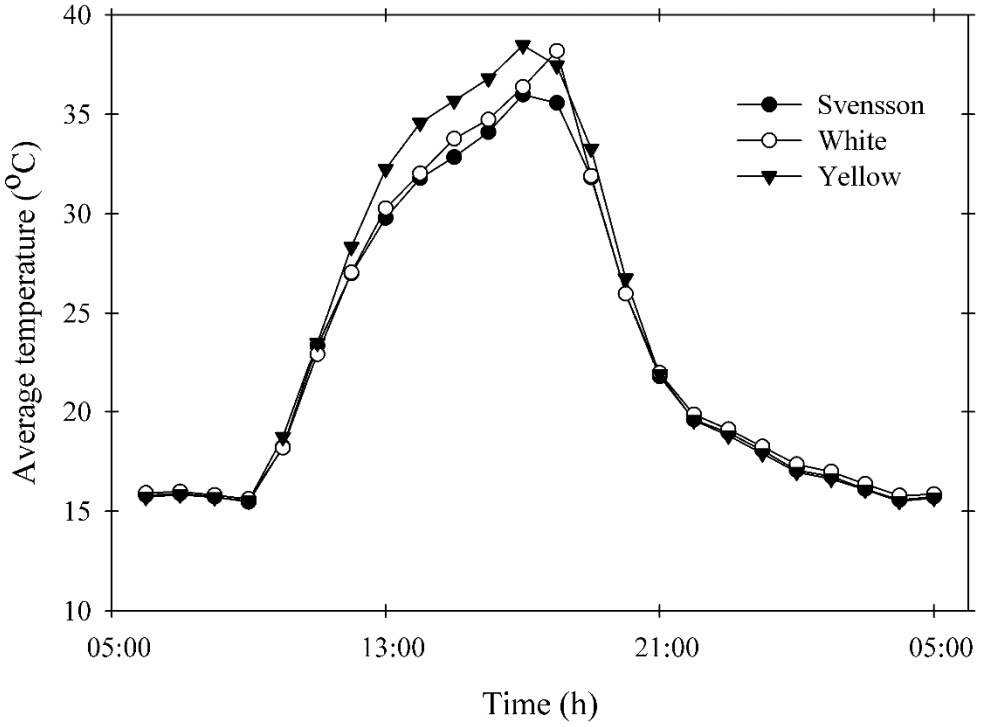

Svensson
White
Yellow
a
b
b
Plant height (cm)
Time (weeks)
0
10
20
30
40
50
0
1
2
3
4
5

Fig 3.


40
35
30
25
20
15
10
Average temperature (°C)
05:00
13:00
21:00
05:00
Time (h)
Svensson
White
Yellow